Patent APPLICATION
IN THE UNAITED STATES PATENT AND TRADEMARK OFFICE
In re application of: Eiji TSURU, et al.
Application No.: 10/526,898 **Group Art Unit: 1626**
Filed: March 7, 2005 **Examiner: JOSEPH R. Kosack**

For: CRYSTAL FOR ORAL SOLID DRUG AND ORAL SOLID DRUG FOR DYSURIA TREATMENT CONTAINING THE SAME

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
Washington, D.C. 20231
Sir:

I, Eiji Tsuru, declare and state as follows.

I am a citizen of Japan residing in Nagano-ken, Japan.

I graduated and got a master's degree in the chemistry (organic chemistry) from Osaka City University, Graduate School of Science in March of 1998.

From April of 1998, I have been employed by KISSEI PHARMACEUTICAL CO., LTD, and I have been engaged in studies on the analytical development, and on the determination of the physicochemical properties of a chemical compound, pharmaceutically acceptable salts thereof and crystalline forms thereof, and so on.

I am one of co-inventors of the present patent application, so, I am familiar with the invention thereof.

The Examiner stated in the Office Action dated July 27, 2006 as follows:

"Williamson specifically teaches to make a heated supersaturated solution of the material to be crystallized, and to cool the solution slowly to room temperature without disturbing the solution after crystallization has started in order to generate crystals" (lines 2-5 on page 4)

"if the person of ordinary skill in the art would crystallize the KMD-3213 generated by Yamagishi et al. or Kitazawa et al. with the addition of the guidance supplied by Williamson, that the person of ordinary skill in the art would generate the claimed invention" (lines 5-9 on page 4)

However, I consider that even if the person of ordinary skill in the art refer the prior art of Yamagishi et al. or Kitazawa et al. together with the guidance supplied by Williamson, the person of ordinary skill in the art would not generate the claimed invention.

In order to demonstrate that the person of ordinary skill in the art would not generate the present α-form crystal of KMD-3213 from Kitazawa et al. (USPN 5,387,603) or Yamagishi et al. (JP07-330726A) together with Williamson (Macroscale and Microscale Organic Experiments 1999, pages 39 and 48-50), I and Mr. Kobayashi who is one of chemists in the central laboratory of our company performed some experiments and I will explain the results of said experiments in detail as follows.

The Examiner stated regard to the guidance of Williamson (hereinafter defined as Williamson' s guidance) that "Williamson teaches the gradual cooling of a heated saturated solution to room temperature to induce crystallization. See pages 48-50." (lines 13-14 on page 6)

On the other hand the Examiner stated regarding to the Williamson' s guidance as that "to cool the solution slowly to room temperature without disturbing the solution after crystallization has started in order to generate crystals".

(lines 3-5 on page 4)

However, this guidance is not regard to the procedure of the crystallization, but regard to the procedure of after the crystallization has started in order to form large crystals. That is, Williamson taught as follows:

"Once it is ascertained that crystallization has started, the solution must be cooled slowly without disturbing the container in order that large crystals can form." (lines 6-8 on page 49)

Williamson only taught regard to the procedure of the crystallization as follows:

"Once it has been ascertained that the hot solution is saturated with the compound just below the boiling point of the solvent, it is allowed to cool slowly to room temperature crystallization should begin immediately. If it does not, add a seed crystal or scratch the inside of the tube with a glass rod at the liquid-air interface." (from line 3 from the bottom on page 48 to line 2 on page 49)

That is, it is clear that the Williamson's guidance regard to the crystallization are (a) cool the hot solution slowly to room temperature, and (b) if the crystallization does not begin, add a seed crystal to the solution. I consider that the Examiner maybe misread the Williamson's guidance.

I will explain that the person of ordinary skill in the art would not generate the present α-form crystal of KMD-3213 from Kitazawa et al. or Yamagishi et al. whatever, even if they refer said Williamson's guidance (a) and (b) above.

First, with regard to Kitazawa et al., Kitazawa et al. disclosed the process for the production of an indoline compound in Example 1 as follows.

"To a mixture of trifluoroacetic acid (0.2 ml) and methylene chloride (0.2ml) was added a solution of 1-acetyl-5-[2-[N-tert-butoxycarbonyl-2-(2-ethoxyphenoxy)ethylamino]propyl]-indoline-7-carboxamide (40 mg) in methylene chloride (0.2 ml) with stirring under ice cooling, and the mixture was stirred at room temperature for 1.5 hours. To the reaction mixture was added a saturated aqueous sodium bicarbonate solution, and the mixture was extracted with methylene chloride. The extract was dried over anhydrous magnesium sulfate. The solvent was concentrated under reduced pressure, and the residue was purified by medium pressure liquid column chromatography on silica gel using a mixture of methylene chloride and methanol (10/1) as eluent to give 33 mg of 1-acetyl-5-[2-[2-(2-ethoxyphenoxy)ethylamino]propyl]-indoline-7-carboxamide as an amorphous powder."

And, Kitazawa et al. also disclosed main peaks of the IR spectrum of KMD-3213 in the Table of Example 2 as compound No. 40 at column 49 as follows.

"IR (cm^{-1}): 3388(NH, OH), 3202(NH), 1637(C=O)"

In order to confirm that Kitazawa et al. did not prepare the present α-form crystal at all, I performed a re-examination of the Example 1 of Kitazawa et al. using a pure KMD-3213 prepared by the improved method (The previous declaration of mine dated June 6, 2006, the fifth full paragraph on page 3) as a KMD-3213 purified by a liquid column chromatography in Example 1 of Kitazawa et al. as follows.

Experiment 1

Re-examination of the Example 1 of Kitazawa et al. (1): TRPB0139

0.5 g of a pure KMD-3213 was dissolved in 27.5 ml of a mixture of methylene chloride and methanol (10/1) and the solvent was evaporated under reduced pressure on the water bath at 33 °C. The residual amorphous solid was collected and was determined

infrared spectrum (IR) of said solid according to the KBr disk method.

Results:

Main peaks of the infrared spectrum (KBr) of said solid were identical to that of Kitazawa et al. For the reference, the infrared chart thereof is attached as Fig. 1.

Experiment 2

Re-examination of the Example 1 of Kitazawa et al. (2): TRPB0134

0.5 g of a pure KMD-3213 was dissolved in 27.5 ml of a mixture of methylene chloride and methanol (10/1) and the solvent was evaporated under reduced pressure on the water bath at 20 °C and then the residue was heated under reduced pressure at 50 °C for two hours. The residual crystalline solid was collected and was determined infrared spectrum (IR) of said solid according to the KBr disk method. Furthermore, I carried out an analysis X-ray powder diffraction of said crystal.

Results:

Main peaks of the infrared spectrum (KBr) of said solid were identical to that of Kitazawa et al. too. For the reference, the infrared chart thereof is attached as Fig. 2.

Furthermore, the X-ray powder diffraction pattern of said crystal exhibits that of the β-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Fig. 3.

From the results, it is sure that Kitazawa et al. did not prepare the present α-form crystal of KMD-3213, but prepared an amorphous or the β-form crystal of KMD-3213.

Kitazawa et al. only disclose as a solvent for the purification of crude product a mixture of methylene chloride and methanol (10/1) using as an eluent for a column chromatography.

Then, I performed recrystallization of KMD-3213 by using said mixture of methylene chloride and methanol (10/1) as a re-crystallizing solvent. As the results, I confirmed that it cannot be performed recrystallization because said solvent freely solves a crude crystal of KMD-3213.

Therefore, even if the person of ordinary skill in the art referred Kitazawa et al. together with Williamson, they would not generate the present α-form crystal of KMD-3213 whatever.

Accordingly, it is clear that Kitazawa et al. only taught the β-form crystal of KMD-3213 and neither taught nor suggested the present α-form crystal of KMD-3213 whatever.

Second, with regard to Yamagishi et al., Mr. Kobayashi who is one of chemists in the central laboratory of our company performed some experiments.

For the reference, I will attach Mr. Kobayashi's EXAMINATION REPORT as ATTACHMENT 1.

Yamagishi et al. disclosed the process for the recrystallization of crude crystal of KMD-3213 using ethyl acetate as a re-crystallizing solvent in Reference Example 30 as follows.

"A precipitated crystal was filtered and was dissolved in 1000 ml of ethyl acetate, and the solution was dried with anhydrous magnesium sulfate. After the solvent was distilled off under reduced pressure the residue was dissolved in 360 ml of ethyl acetate at 70 °C and the solution was allowed to stand at the room temperature to obtain 52.1 g of (R)-(-)-1-(3-Hydroxypropyl)-5-[2-[2-[2-(2,2,2,-trifuluoloethoxy)phenoxy]ethylamino]propyl]-indoline-7-carboxamide wherein the melting point thereof is 107-108 °C."

And, Yamagishi et al. also disclosed main peaks of the IR spectrum of KMD-3213 which are identical to that of Kitazawa et al. as follows.

"IR (KBr): νNH, OH 3388 cm^{-1}, νNH 3202 cm^{-1} and νC=O 1637 cm^{-1}"

Mr. Kobayashi performed a re-examination of the Reference Example 30 of Yamagishi et al. and he reported in his EXAMINATION REPORT as follows:

"a crude crystal of KMD-3213 of Yamagishi et al. is relatively low purity, and so in a recrystallization using ethyl acetate as a recrystallizing solvent, since the crystallization did not begin at the point of the saturated solution was cooled to room temperature it is needed to add a seed crystal to the solution, and thus the obtained crystal is the β-form crystal." (the first full paragraph on page 9) And,

"it can be obtained the β-form crystal of KMD-3213 by re-crystallizing of crude crystal of KMD-3213 of Yamagishi et al. using ethyl acetate as a re-crystallizing solvent according to the Williamson's guidance." (the fifth full paragraph on page 3)

That is, Mr. Kobayashi confirmed that even if performed recrystallization of KMD-3213 of Yamagishi et al, using ethyl acetate as a re-crystallizing solvent and refer to the Williamson's guidance, it cannot be obtained the present α-form crystal but the β-form crystal.

However Yamagishi et al. did not disclose an addition of a seed crystal in Reference Example 30, it is however considered that they merely omitted the description of that step as a usual and routine step.

Mr. Kobayashi considered from the results of a re-examination of Reference Example 30 of Yamagishi et al. as follows:

"in case of a re-crystallizing of crude crystal of KMD-3213 of Yamagishi et al., any or some factor selected from the factors of (a) a seeding of a seed crystal, (b) impurities of contained in a crude crystal of KMD-3213 prepared by the Yamagishi et al. (hereinafter defined as impurities of Yamagishi et al.) and (c) a temperature of a saturated solution at beginning of crystallization or a temperature of a saturated solution at a seeding of a seed crystal (herein after defined as a temperature of beginning of crystallization) maybe affect on crystallization of polymorphs of KMD-3213" (paragraph bridging pages 3 and 4)

And in order to investigate any factor would affect on crystallization of polymorphs of KMD-3213 in case of the recrystallization of KMD-3213 of Yamagishi et al., he performed some comparative examinations.

Mr. Kobayashi summarized the results of said comparative examinations as follows:

No.	Code	Impurities of Yamagishi et al.	A seed crystal	A temperature of beginning of crystallization	Results (crystal form)
(1)	QAJG0027 -m2	none	β-form	About 19 ℃	β-form
(2)	QAJG0023 -eve	none	β-form	About 29 ℃	α-form
(3)	QAJG0024 -3-eve	5%	β-form	About 18 ℃	β-form
(4)	QAJG0024 -1-eve	5%	β-form	About 32 ℃	β-form

Furthermore, Mr. Kobayashi confirmed that in case of the recrystallization of pure KMD-3213 by using ethyl acetate as a re-crystallizing solvent, a temperature of beginning of crystallization that is a temperature of a saturated solvent at

seeding the seed crystal may most affect on crystallization of polymorphs. That is,
(1) if a temperature of beginning of crystallization is below room temperature (below about 20 °C) it can be obtained the β-form crystal (comparative examination (1): QAJG0027-m2), and
(2) if said temperature is high temperature (up to about 30 °C) it can be obtained the α-form crystal even if the seed crystal is the β-form crystal of KMD-3213 (comparative examination (2): QAJG0023-eve).
(the first full paragraph and the second full paragraph on page 10)

And Mr. Kobayashi further confirmed that in case of the recrystallization of crude crystal of KMD-3213 contained impurities of Yamagishi et al. by using ethyl acetate as a re-crystallizing solvent, impurities of Yamagishi et al. may most affect on crystallization of polymorphs and thus regardless a degree of a temperature of beginning of crystallization it can be obtained the β-form crystal of KMD-3213. That is,
(3) if a temperature of beginning of crystallization is below room temperature (below about 20 °C) it can be obtained the β-form crystal (comparative examination (3): QAJG0024-3-eve), and
(4) even if said temperature is high temperature (up to about 30 °C) it can be obtained the β-form crystal (comparative examination (4): QAJG0024-1-eve).
(the third full paragraph and the fourth full paragraph on page 10)

And further, Mr. Kobayashi concluded as follows:
"in case of the recrystallization of crude crystal of KMD-3213 of Yamagishi et al. by using ethyl acetate as a re-crystallizing solvent, since impurities of Yamagishi et al. may most affect on crystallization of polymorphs and thus regardless a degree of a temperature of beginning of crystallization it can be obtained the β-form crystal of KMD-3213" (paragraph bridging pages 10 and 11)

As stated above, regard to the procedure of the crystallization Williamson taught (a) (the solution) is allowed to cool slowly to room temperature and (b) (if the crystallization did not begin) add a seed crystal, that is, Williamson taught a method that to start the crystallization add a seed crystal to the solution after cooling the saturated solution to room temperature.

And, said method is just a method to generate the β-form crystal of KMD-3213, in case of the recrystallization of KMD-3213 by using ethyl acetate as a re-crystallizing solvent stated by Mr. Kobayashi.

And furthermore, as stated above, Mr. Kobayashi concluded that in case of the recrystallization of crude crystal of KMD-3213 of Yamagishi et al. by using ethyl acetate as a re-crystallizing solvent, impurities of Yamagishi et al. may most affect on crystallization of polymorphs and thus regardless a degree of a temperature of beginning of crystallization it can be obtained the β-form crystal of KMD-3213.

Thus, it is sure that even if the person of ordinary skill in the art referred Yamagishi et al. together with Williamson, they would not generate the present α-form crystal of KMD-3213 whatever.

It is clear that Yamagishi et al. only taught the β-form crystal of KMD-3213 and neither taught nor suggested the present α-form crystal of KMD-3213 whatever.

Furthermore, Yamagishi et al. prepared KMD-3213 as a synthetic intermediate for producing the indole compound disclosed in Example 1. That is, KMD-3213 of Yamagishi et al. is never a subject compound of the invention.

Accordingly, the person of ordinary skill in the art would never have a motivation to attempt to modify the Yamagishi et al. procedure by the guidance of Williamson to generate the present α-form crystal of KMD-3213 whatever.

It is well known that polymorphism is often affected by a trace amount of impurities. (Masaki Okamoto et al., Journal of Chemical Engineering of Japan, vol. 37, No. 10, pp. 1224-1231, 2004; ATTACHMENT 2)

Furthermore, it is well known that polymorphism is often affected by a seeding of a seed crystal and a temperature of a solution at seeding. (R. J. Davey et al., J. Am. Chem. Soc., 1997, 119, 1767-1772; ATTACHMENT 3) In that document, R. J. Davey et al. stated that pure α was prepared by seeding a 20 g/L aqueous solution as 18 °C, pure β was prepared by unseeded crystallization of a 35 g/L aqueous solution as 38 °C. (lines 12-14 on page 1769)

Additionally, the present α-form crystal of KMD-3213 is the most preferable for the crystal for an oral solid medicament as regards stability and hygroscopicity (lines 16-19 on page 6 of the present specification) and the present invention is related to that findings. And, both of Kitazawa et al. and Yamagishi et al. never disclosed nor suggested said superiority of the present α-form crystal of KMD-3213 whatever.

I believe that the present invention is novel and is never obviously from the prior art whatever.

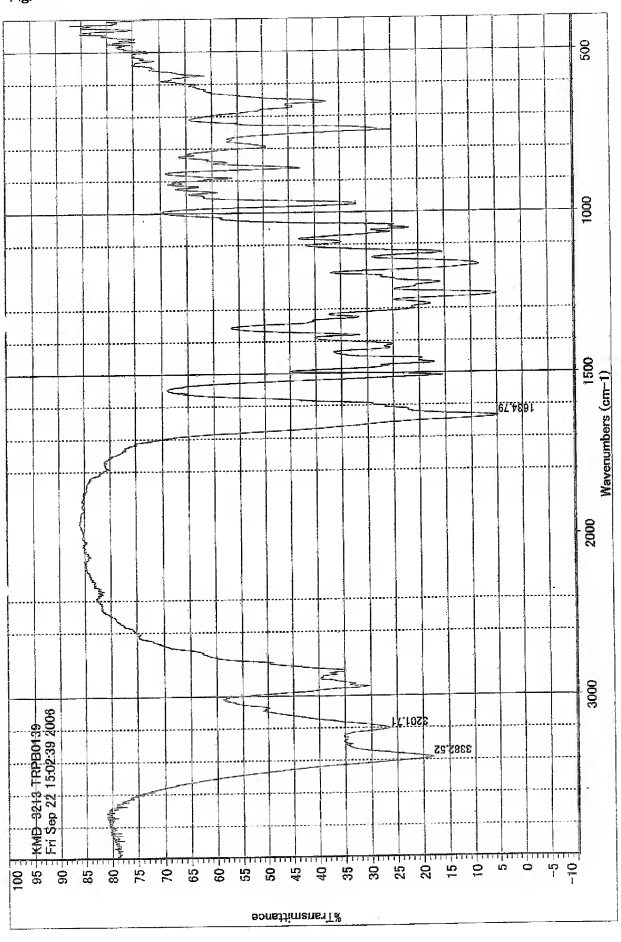
I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code

and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dec 18, 2006
Date

Eiji Tsuru
Eiji Tsuru

Fig. 1


KMD 9218 TRPB0139
Fri Sep 22 15:02:39 2006
%Transmittance
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3382.52
2201.71
1684.79

Fig. 2

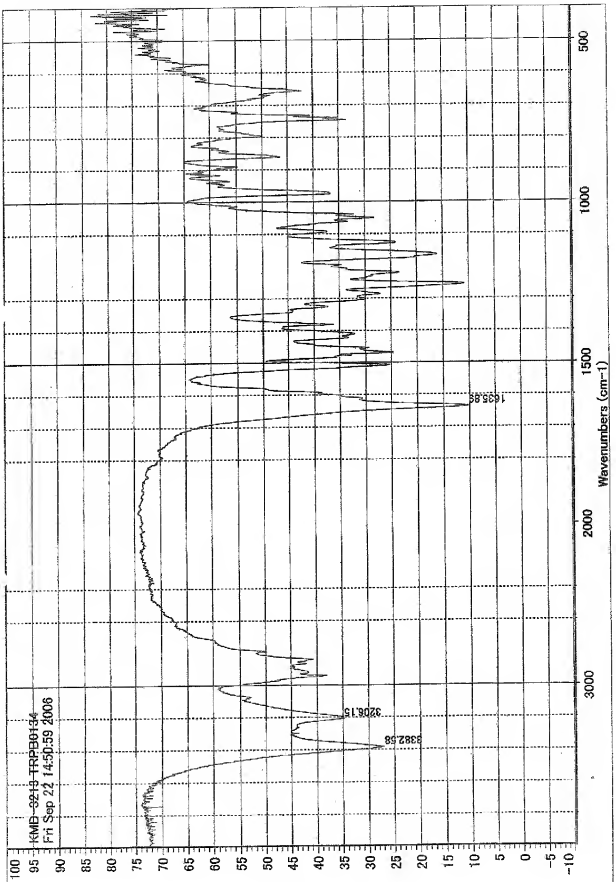
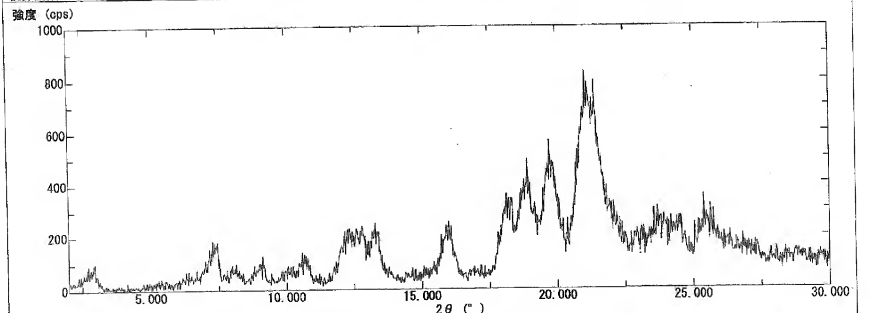

%Transmittance
Wavenumbers (cm-1)
KMD-3213-TRPB0134
Fri Sep 22 14:50:59 2006
3382.58
3206.15
1635.85

Fig. 3

生データ

ｻﾝﾌﾟﾙ名	: KMD-3213	ﾌｧｲﾙ	: 060912d03.raw	ｺﾒﾝﾄ	: TRPB0134
測定者	: E.Tsuru	測定日	: 12-September-06 15:22:31		
ﾒﾓ	: CHCl3/MeOH(10:1)濃縮 0.2mm				
X線	: Cu / 40 kV / 40 mA				
ｺﾞﾆｵﾒｰﾀ	: Ultima+ 水平ｺﾞﾆｵﾒｰﾀ				
ｱﾀｯﾁﾒﾝﾄ	: 6ｻﾝﾌﾟﾙﾁｪﾝｼﾞｬｰB型				
試料番号	: 3				
試料回転	: 回転	回転速度	: 120.000 rpm		
ﾌｨﾙﾀ	: 不使用	発散ｽﾘｯﾄ	: 1/2°	ﾓﾉｸﾛ受光ｽﾘｯﾄ	: 0.45mm
ｲﾝｼﾞｹｰﾄﾓﾉｸﾛ	: 不使用	発散縦制限ｽﾘｯﾄ	: 10mm		
ｶｳﾝﾀﾓﾉｸﾛﾒｰﾀ	: 固定ﾓﾉｸﾛﾒｰﾀ	散乱ｽﾘｯﾄ	: 1/2°		
ｶｳﾝﾀ	: ｼﾝﾁﾚｰｼｮﾝｶｳﾝﾀ	受光ｽﾘｯﾄ	: 0.15mm		
走査ﾓｰﾄﾞ	: 連 続	ｽｷｬﾝ ｽﾋﾟｰﾄﾞ	: 4.000 °/min.	ｻﾝﾌﾟﾘﾝｸﾞ幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 2.000 ～ 30.000 °	θｵﾌｾｯﾄ	: 0.000 °
積算回数	: 1				



ATTACHMENT 1

EXAMINATION REPORT

INTRODUCTION

I, Hiroaki Kobayashi, am a citizen of Japan residing in Nagano-ken, Japan. I graduated and got a master's degree in the Engineering (Materials Science and Chemical Engineering) from Yokohama National University, Graduate School of Engineering in March of 1993. From April of 1993, I have been employed by KISSEI PHARMACEUTICAL CO., LTD, and I have been engaged in studies on the research and development of a chemical compound.

In order to confirm that whether the present α-form crystal of KMD-3213 is produced or not by recrystallization of crude crystal of KMD-3213 prepared by a procedure of Reference Example 30 of Yamagishi et al. (JP07-330726A) (hereinafter defined as KMD-3213 of Yamagishi et al.) using ethyl acetate as a recrystallizing solvent referring Williamson (Macroscale and Microscale Organic Experiments 1999, pages 39 and 48-50), I performed some experiments and I will explain the results of said experiments in detail as follows.

Yamagishi et al. disclosed the process for the production of KMD-3213 and the process for the recrystallization of said crude crystal of KMD-3213 using ethyl acetate as a recrystallizing solvent in Reference Example 30 as follows.

"To a solution of 70.9 g of (R)-(-)-1-(3-Benzyloxypropyl)-5-[2-[2-[2-(2,2,2,-trifuluoloethoxy)-phenoxy] ethylamino] propyl] indoline-7-carboxamide in 560 ml of ethanol, 7.1 g of 10% palladium carbon and 292 ml of 1N-hydrochrolic acid were added under ice-cooling with stirring and the mixture was reacted under a hydrogen atmosphere for 3 hours. After completion of the reaction, the catalyst was filtered off and the filtrate was

concentrated under reduced pressure. To the residue 1000 ml of ion-exchanged added and the solution was washed 3 times with 250 ml of ethyl acetate. The aqueous solution was adjusted to pH 8 by adding 250 ml of 10% sodium carbonate aqueous solution under ice-cooling with stirring and the solution was stirred for 18 hours at the room temperature. A precipitated crystal was filtered and was dissolved in 1000 ml of ethyl acetate, and the solution was dried over anhydrous magnesium sulfate. After the solvent was distilled off under reduced pressure the residue was dissolved in 360 ml of ethyl acetate at 70 ℃ and the solution was allowed to stand at room temperature to obtain 52.1 g of (R)-(-)-1-(3-Hydroxypropyl)-5-[2-[2-[2-(2,2,2,-trifuluolo ethoxy)phenoxy] ethylamino] propyl] indoline-7-carboxamide wherein the melting point thereof is 107-108 ℃."

I prepared (R)-(-)-1-(3-Benzyloxypropyl)-5-[2-[2-[2-(2,2,2,-trifuluoloethoxy)-phenoxy] ethylamino] propyl] indoline-7-carboxamide as a starting compound of Reference Example 30 according to the process of Figure 1, and I performed a re-examination of the Reference Example 30 by employing said compound.

On the other hand, Williamson disclosed a usual and basic procedure for crystallization and stated regard to crystallizing as follows:

(1) "Once it has been ascertained that the hot solution is saturated with the compound just below the boiling point of the solvent, it is allowed to cool slowly to room temperature. Crystallization should begin immediately. If it does not, add a seed crystal or scratch the inside of the tube with a glass rod at the liquid-air interface." (from line 3 from the bottom on page 48 to line 2 on page 49)

(2) "Once it is ascertained that crystallization has started, the solution must be cooled slowly without disturbing the container in order that large crystals can form." (lines 6-8 on page 49)

However, second guidance is not regard to the procedure of the crystallization, but regard to the procedure of after the crystallization has started in order to form large crystals.

Accordingly, Williamson's guidance regard to the crystallization is only the first guidance and said guidance having two points of (a) hot solution is allowed to cool slowly to room temperature and (b) if the crystallization does not begin, add a seed crystal to the solution.

Therefore, I referred said two points as the Williamson's guidance on re-examination of Reference Example 30 of Yamagishi et al.

Furthermore, I employed the β-form crystal of KMD-3213 as a seed crystal. Because Mr. Tsuru confirmed that Kitazawa et al. would be prepared the β-form crystal of KMD-3213, thus I consider that Yamagishi et al. maybe stocked the β-form crystal of KMD-3213 at the time said invention was made.

As the results of said re-examination of the Reference Example 30 of Yamagishi et al., I confirmed that it can be obtained the β-form crystal of KMD-3213 by recrystallizing of crude crystal of KMD-3213 of Yamagishi et al. using ethyl acetate as a recrystallizing solvent according to the Williamson's guidance.

And from the results that by recrystallizing of crude crystal of KMD-3213 of Yamagishi et al. using ethyl acetate it cannot be obtained the α-form crystal of KMD-3213, but can be obtained the β-form crystal of KMD-3213, I considered that in case of a recrystallizing of crude crystal of KMD-3213 of Yamagishi et al., any or some factor selected from the factors of (a) a seeding of a seed crystal, (b) impurities of contained in a crude crystal of KMD-3213 prepared by the Yamagishi et al. (hereinafter defined as impurities of Yamagishi et al.) and (c) a temperature

of a saturated solution at beginning of crystallization or a temperature of a saturated solution at a seeding of a seed crystal (herein after defined as a temperature of beginning of crystallization) maybe affect on crystallization of polymorphs of KMD-3213.

Then, in order to investigate any factor would affect on crystallization of polymorphs of KMD-3213 in case of the recrystallization of KMD-3213 of Yamagishi et al. by using ethyl acetate as a recrystallizing solvent, I performed some comparative examinations of a recrystallization of KMD-3213 using ethyl acetate as a recrystallizing solvent, by employing KMD-3213 prepared by a procedure of Figure 2 (herein after defined as the improved method) as a pure KMD-3213 and a concentrated residue of the filtrate of the recrystallization of a crude crystal of KMD-3213 of Yamagishi et al. in the above re-examination of Reference Example 30 of Yamagishi et al. as impurities of Yamagishi et al..

I will explain the Experiments and the results thereof in detail as follows.

EXPERIMENTS

Experiment 1

Re-examination of the Reference Example 30 of Yamagishi et al.: QAJG0014-1

To a solution of 12.45 g of (R)-(-)-1-(3-Benzyloxypropyl)-5-[2-[2-[2-(2,2,2,-trifuluoloethoxy)-phenoxy] ethylamino] propyl] indoline-7-carboxamide in 98 ml of ethanol, 1.25 g of 10% palladium carbon and 51.0 ml of 1N-hydrochrolic acid were added under ice-cooling with stirring and the mixture was reacted under a hydrogen atmosphere for 3 hours.

After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. To the residue 170 ml of ion-exchanged added and the solution was washed 3 times with 50 ml of ethyl acetate. The aqueous solution was adjusted to pH 8 by adding 5.63 g of sodium carbonate under ice-cooling with stirring and the solution was stirred for 18 hours at room temperature. A precipitated crystal was filtered and was dissolved in 170 ml of ethyl acetate, and the solution was dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to obtain a crude crystal of KMD-3213 of 12.45 g.

12.45 g of the crude crystal of KMD-3213 above obtained was dissolved in about 74 ml of ethyl acetate with heating on a water bath at about 70 ℃ and the solution was cooled to room temperature (about 17.5 ℃; the temperature of the solution is about 21.6 ℃). Since the crystallization did not begin, a seed crystal was added to the solution according to the Williamson' s guidance and then the solution was allowed to stand at room temperature over night. The precipitated crystals were collected by filtration (7.93 g).

It was carried out an analysis X-ray powder diffraction of said crystal and it further determined infrared spectrum (IR) of said crystal according to the KBr disk method.

Results:

The X-ray powder diffraction pattern of said crystal exhibits that of the β-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Figure 3.

And the main peaks of the infrared spectrum (IR) of said crystal are identical to that of Yamagishi et al. For the reference, the infrared chart thereof is attached as Figure 4.

From the results, it is sure that by recrystallization of crude crystal of KMD-3213 of Yamagishi et al. using ethyl acetate

as a recrystallizing solvent and according to Williamson's guidance it can be obtained the β-form crystal of KMD-3213.

Experiment 2

Comparative examination of recrystallization of KMD-3213(1): QAJG0027-m2

5.0 g of a pure KMD-3213 was dissolved in about 60 ml of ethyl acetate with heating and the solution was cooled to that the temperature of the solution is about 19 ℃ and a seed crystal was added to the solution and then the solution was allowed to stand at room temperature over night. The precipitated crystals were collected by filtration.

It was carried out an analysis X-ray powder diffraction of said crystal and it further determined infrared spectrum (IR) of said crystal according to the KBr disk method.

Results:

The X-ray powder diffraction pattern of said crystal exhibits that of the β-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Figure 5.

And the main peaks of the infrared spectrum (IR) of said crystal are identical to that of Yamagishi et al. For the reference, the infrared chart thereof is attached as Figure 6.

Experiment 3

Comparative examination of recrystallization of KMD-3213(2): QAJG0023-eve

5.0 g of a pure KMD-3213 was dissolved in about 60 ml of ethyl acetate with heating and the solution was cooled to that the temperature of the solution is about 29 ℃ and a seed crystal was added to the solution and then the solution was allowed to

stand at room temperature over night. The precipitated crystals were collected by filtration.

It was carried out an analysis X-ray powder diffraction of said crystal and it further determined infrared spectrum (IR) of said crystal according to the KBr disk method.

Results:

The X-ray powder diffraction pattern of said crystal exhibits that of the α-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Figure 7.

And the main peaks of the infrared spectrum (IR) of said crystal are that of the α-form crystal. For the reference, the infrared chart thereof is attached as Figure 8.

Experiment 4

Comparative examination of recrystallization of KMD-3213 (3): QAJG0024-3-eve

A mixture of 5.0 g of a pure KMD-3213 and 0.25 g (5%) of impurities of Yamagishi et al. was dissolved in about 60 ml of ethyl acetate with heating and the solution was cooled to that the temperature of the solution is about 18 ℃ and a seed crystal was added to the solution and then the solution was allowed to stand at room temperature over night. The precipitated crystals were collected by filtration.

It was carried out an analysis X-ray powder diffraction of said crystal and it further determined infrared spectrum (IR) of said crystal according to the KBr disk method.

Results:

The X-ray powder diffraction pattern of said crystal exhibits that of the β-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Figure 9.

And the main peaks of the infrared spectrum (IR) of said crystal are identical to that of Yamagishi et al. For the reference, the infrared chart thereof is attached as Figure 10.

Experiment 5

Comparative examination of recrystallization of KMD-3213 (4): QAJG0024-1-eve

A mixture of 5.0 g of a pure KMD-3213 and 0.25 g (5%) of impurities of Yamagishi et al. was dissolved in about 60 ml of ethyl acetate with heating and the solution was cooled to that the temperature of the solution is about 32 ℃ and a seed crystal was added to the solution and then the solution was allowed to stand at room temperature over night. The precipitated crystals were collected by filtration.

It was carried out an analysis X-ray powder diffraction of said crystal and it further determined infrared spectrum (IR) of said crystal according to the KBr disk method.

Results:

The X-ray powder diffraction pattern of said crystal exhibits that of the β-form crystal. For the reference, I showed the X-ray powder diffraction pattern of said crystal in Figure 12.

And the main peaks of the infrared spectrum (IR) of said crystal were identical to that of Yamagishi et al. For the reference, the infrared chart thereof is attached as Figure 13.

Summary of the results of the comparative examinations:

No.	Code	Impurities of Yamagishi et al.	A seed crystal	A temperature of beginning of crystallization	Results (crystal form)

(1)	QAJG0027 -m2	none	β-form	About 19 ℃	β-form
(2)	QAJG0023 -eve	none	β-form	About 29 ℃	α-form
(3)	QAJG0024 -3-eve	5%	β-form	About 18 ℃	β-form
(4)	QAJG0024 -1-eve	5%	β-form	About 32 ℃	β-form

RESULTS AND DISCUSSION

As the results of the re-examination of the Reference Example 30 of Yamagishi et al., I confirmed that a crude crystal of KMD-3213 of Yamagishi et al. is relatively low purity, and so in a recrystallization using ethyl acetate as a recrystallizing solvent, since the crystallization did not begin at the point of the saturated solution was cooled to room temperature it is needed to add a seed crystal to the solution, and thus the obtained crystal is the β-form crystal.

From the results, it can be assumed that any or some factor selected from the factors of (a) a seeding of a seed crystal, (b) impurities of Yamagishi et al. and (c) a temperature of beginning of crystallization may affect on crystallization of polymorphs of KMD-3213 of Yamagishi et al. using ethyl acetate as a recrystallizing solvent. Then, in order to investigate any factor would affect on crystallization of polymorphs of KMD-3213 of Yamagishi et al. by using ethyl acetate as a recrystallizing solvent, I performed some comparative examinations of a recrystallization of KMD-3213 using ethyl acetate as a recrystallizing solvent, by employing a pure KMD-3213 prepared by the improved method) and impurities of Yamagishi et al. which is a concentrated residue of the filtrate of the recrystallization of a crude crystal of KMD-3213 of Yamagishi et al.

As the results of the comparative examinations, I confirmed that in case of the recrystallization of pure KMD-3213 by using ethyl acetate as a recrystallizing solvent, a temperature of beginning of crystallization may most affect on crystallization of polymorphs.

That is, (1)if a temperature of beginning of crystallization is below room temperature (below about 20 °C) it can be obtained the β-form crystal (comparative examination (1): QAJG0027-m2), and (2)if said temperature is high temperature (up to about 30 °C) it can be obtained the α-form crystal even if the seed crystal is the β-form crystal of KMD-3213 (comparative examination (2): QAJG0023-eve).

On the other hand, I confirmed that in case of the recrystallization of crude crystal of KMD-3213 contained impurities of Yamagishi et al. by using ethyl acetate as a recrystallizing solvent, impurities of Yamagishi et al. may most affect on crystallization of polymorphs and thus regardless a degree of a temperature of beginning of crystallization it can be obtained the β-form crystal of KMD-3213.

That is, (3)if a temperature of beginning of crystallization is below room temperature (below about 20 °C) it can be obtained the β-form crystal (comparative examination (3): QAJG0024-3-eve), and (4)even if said temperature is high temperature (up to about 30 °C) it can be obtained the β-form crystal (comparative examination (4): QAJG0024-1-eve).

Accordingly, I concluded that in case of the recrystallization of crude crystal of KMD-3213 of Yamagishi et al. by using ethyl acetate as a recrystallizing solvent, since impurities of Yamagishi et al. may most affect on crystallization of polymorphs and thus regardless a degree of a temperature of beginning of crystallization it can be obtained the β-form crystal

of KMD-3213.

That is, it is sure that in case of the recrystallization of crude crystal of KMD-3213 of Yamagishi et al. even if referring the Williamson' s guidance it cannot be obtained the present α-form crystal of KMD-3213, but can be obtained the β-form crystal.

December 13, 2006
Date

Hiroaki Kobayashi
Hiroaki Kobayashi

Figure 1

Synthetic route of KMD-3213 (crude crystal) of Re-examination of Ref. Ex. 30 of JP 07-330726A (Yamagishi et al)

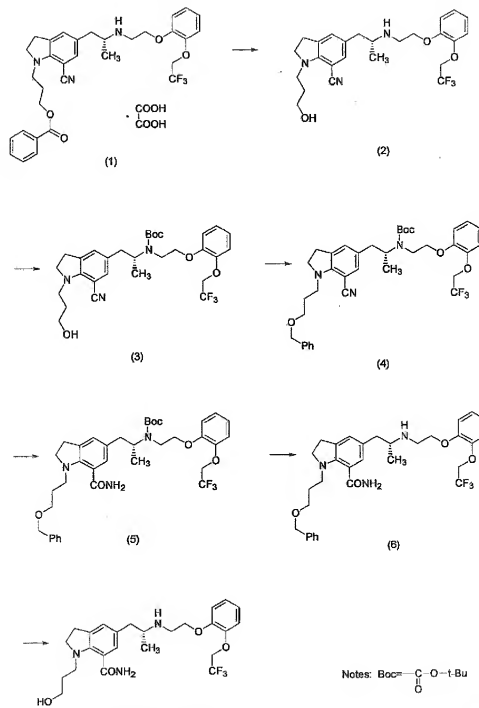


Figure 2

Synthetic route of KMD-3213 (crude crystal) of Re-examination of the present Example 1

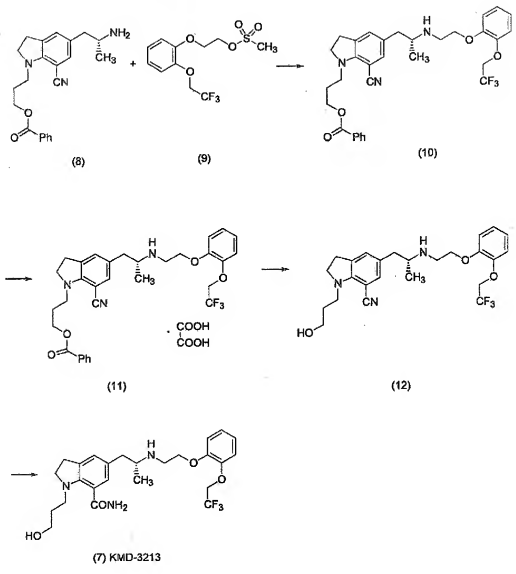
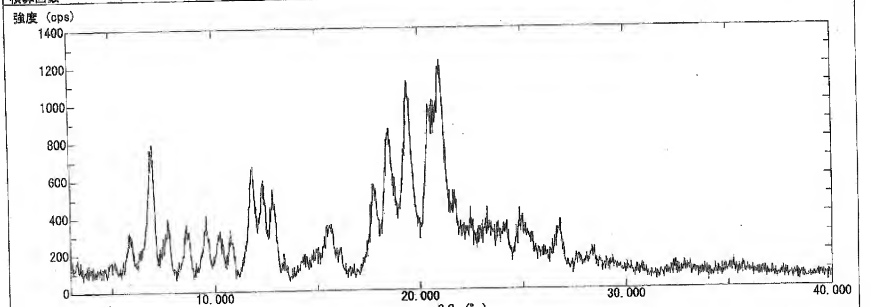


Figure 3

生データ

ｻﾝﾌﾟﾙ名	: KMD-3213	ﾌｧｲﾙ	: 061018d05.raw	ｺﾒﾝﾄ	: QAJG0014-1
測定者	: E.Tsuru	測定日	: 18-October-06 15:38:39		
ﾒﾓ	: 2mm				
X線	: Cu / 40 kV / 40 mA				
ｺﾞﾆｵﾒｰﾀ	: Ultima+ 水平ｺﾞﾆｵﾒｰﾀ				
ｱﾀｯﾁﾒﾝﾄ	: 6ｻﾝﾌﾟﾙﾁｪﾝｼﾞｬｰB型				
試料番号	: 1				
試料回転	: 回転	回転速度	: 120.000 rpm		
ﾌｨﾙﾀ	: 不使用	発散ｽﾘｯﾄ	: 1/2°	ﾓﾉｸﾛ受光ｽﾘｯﾄ	: 0.45mm
ｲﾝｼﾃﾞﾝﾄﾓﾉｸﾛ	: 不使用	発散縦制限ｽﾘｯﾄ	: 10mm		
ｶｳﾝﾀﾓﾉｸﾛﾒｰﾀ	: 固定ﾓﾉｸﾛﾒｰﾀ	散乱ｽﾘｯﾄ	: 1/2°		
ｶｳﾝﾀ	: ｼﾝﾁﾚｰｼｮﾝｶｳﾝﾀ	受光ｽﾘｯﾄ	: 0.15mm		
走査ﾓｰﾄﾞ	: 連 続	ｽｷｬﾝ ｽﾋﾟｰﾄﾞ	: 4.000 °/min.	ｻﾝﾌﾟﾘﾝｸﾞ幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 3.000 ～ 40.000 °	θｵﾌｾｯﾄ	: 0.000 °
積算回数	: 1				



2006-October-18 15:48:19 Page-1

Figure 4

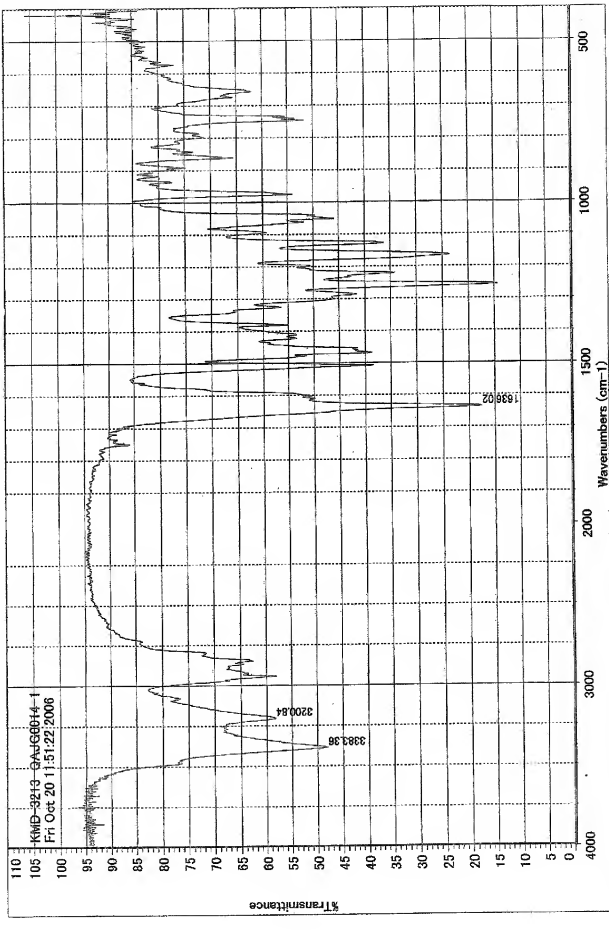
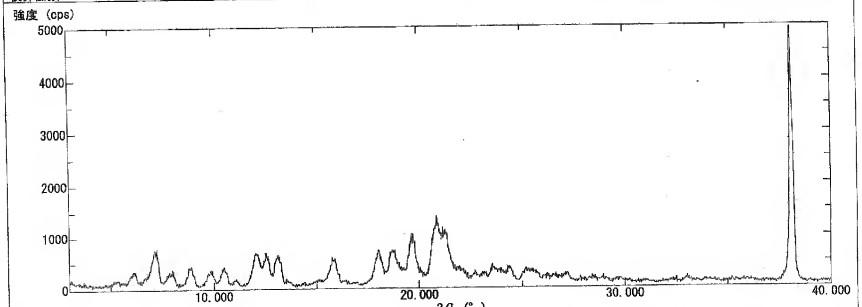

KMD-3213 GAUG60114-1
Fri Oct 20 11:51:22 2006
%Transmittance
Wavenumbers (cm-1)
3383.36
3200.84
1638.02

Figure 5

生データ

ｻﾝﾌﾟﾙ名	: KMD-3213	ﾌｧｲﾙ	: 061109d05.raw	ｺﾒﾝﾄ	: QAJG0027-m2
測定者	: E.Tsuru	測定日	: 09-November-06 15:30:12		
ﾒﾓ	: 0.2mm				
X線	: Cu / 40 kV / 40 mA				
ｺﾞﾆｵﾒｰﾀ	: Ultima+ 水平ｺﾞﾆｵﾒｰﾀ				
ｱﾀｯﾁﾒﾝﾄ	: GｻﾝﾌﾟﾙﾁｪﾝｼﾞｬｰB型				
試料番号	: 5				
試料回転	: 回転	回転速度	: 120.000 rpm		
ﾌｨﾙﾀ	: 不使用	発散ｽﾘｯﾄ	: 1/2°	ﾓﾉｸﾛ受光ｽﾘｯﾄ	: 0.45mm
ｲﾝｼﾃﾞﾝﾄﾓﾉｸﾛ	: 不使用	発散縦制限ｽﾘｯﾄ	: 10mm		
ｶｳﾝﾀﾓﾉｸﾛﾒｰﾀ	: 固定ﾓﾉｸﾛﾒｰﾀ	散乱ｽﾘｯﾄ	: 1/2°		
ｶｳﾝﾀ	: ｼﾝﾁﾚｰｼｮﾝｶｳﾝﾀ	受光ｽﾘｯﾄ	: 0.15mm		
走査ﾓｰﾄﾞ	: 連 続	ｽｷｬﾝ ｽﾋﾟｰﾄﾞ	: 4.000 °/min.	ｻﾝﾌﾟﾘﾝｸﾞ幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 3.000 ～ 40.000 °	θｵﾌｾｯﾄ	: 0.000 °
積算回数	: 1				



2006-November-09 15:39:57 Page-1

Figure 6

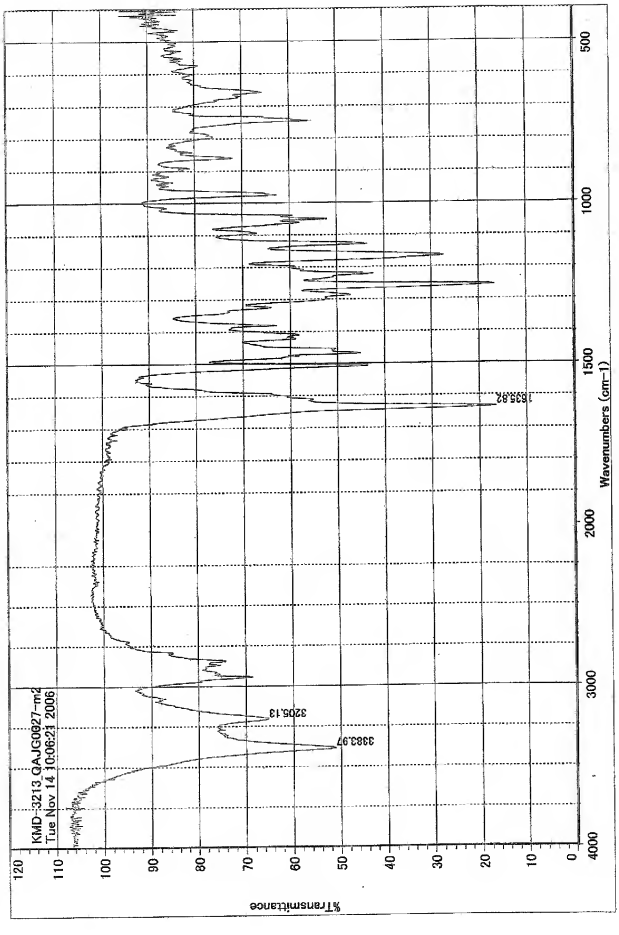
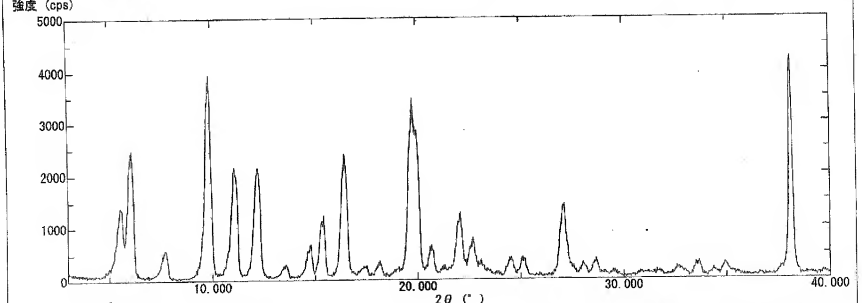

KMD-3213 QAJG0827-m2
Tue Nov 14 10:06:21 2006
%Transmittance
Wavenumbers (cm-1)
3383.97
3205.13
1635.82
4000
3000
2000
1500
1000
500
120
110
100
90
80
70
60
50
40
30
20
10
0

Figure 7

生データ

サンプル名	: KMD-3213	ファイル	: 061109d02.raw	コメント	: QAJG0023-eve
測定者	: E.Tsuru	測定日	: 09-November-06 14:59:33		
メモ	: 0.2mm				
X線	: Cu / 40 kV / 40 mA				
ゴニオメータ	: Ultima+ 水平ゴニオメータ				
アタッチメント	: 6サンプルチェンジャーB型				
試料番号	: 2				
試料回転	: 回転	回転速度	: 120.000 rpm		
フィルタ	: 不使用	発散スリット	: 1/2°	モノクロ受光スリット	: 0.45mm
インデントモノクロ	: 不使用	発散縦制限スリット	: 10mm		
カウンタモノクロメータ	: 固定モノクロメータ	散乱スリット	: 1/2°		
カウンタ	: シンチレーションカウンタ	受光スリット	: 0.15mm		
走査モード	: 連 続	スキャン スピード	: 4.000 °/min.	サンプリング幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 3.000 ～ 40.000 °	θオフセット	: 0.000 °
積算回数	: 1				



2006-November-09 15:09:18 Page-1

Figure 8

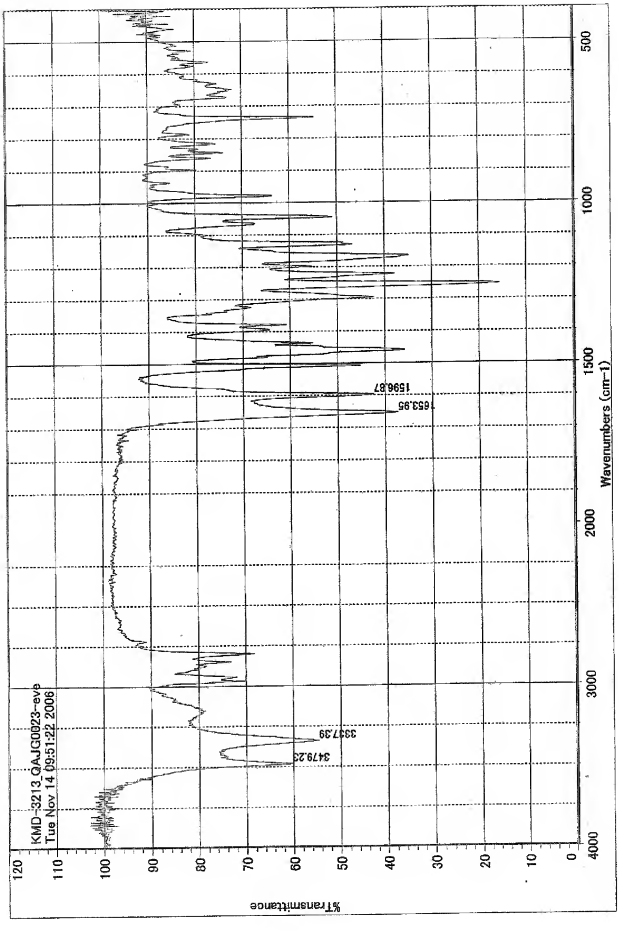

KMD-3213-GAJG0823-evp
Tue Nov 14 09:51:22 2006
%Transmittance
Wavenumbers (cm-1)
3379.23
3337.39
1653.95
1596.87
120
110
100
90
80
70
60
50
40
30
20
10
0
4000
3000
2000
1500
1000
500

Figure 9

生データ

サンプル名	: KMD-3213	ファイル	: 061109d04.raw	コメント	: QAJG0024-3-eve
測定者	: E.Tsuru	測定日	: 09-November-06 15:19:59		
メモ	: 0.2mm				
X線	: Cu / 40 kV / 40 mA				
ゴニオメータ	: Ultima+ 水平ゴニオメータ				
アタッチメント	: 6サンプルチェンジャーB型				
試料番号	: 4				
試料回転	: 回転	回転速度	: 120.000 rpm		
フィルタ	: 不使用	発散スリット	: 1/2°	モノクロ受光スリット	: 0.45mm
インシデントモノクロ	: 不使用	発散縦制限スリット	: 10mm		
カウンタモノクロメータ	: 固定モノクロメータ	散乱スリット	: 1/2°		
カウンタ	: シンチレーションカウンタ	受光スリット	: 0.15mm		
走査モード	: 連 続	スキャン スピード	: 4.000 °/min.	サンプリング幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 3.000 ～ 40.000 °	θオフセット	: 0.000 °
積算回数	: 1				

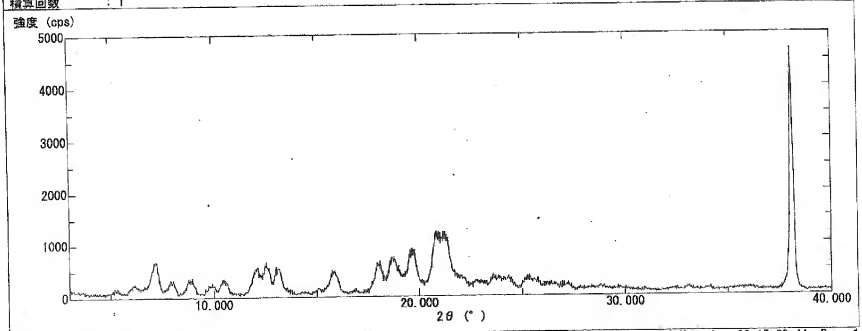


Figure 10

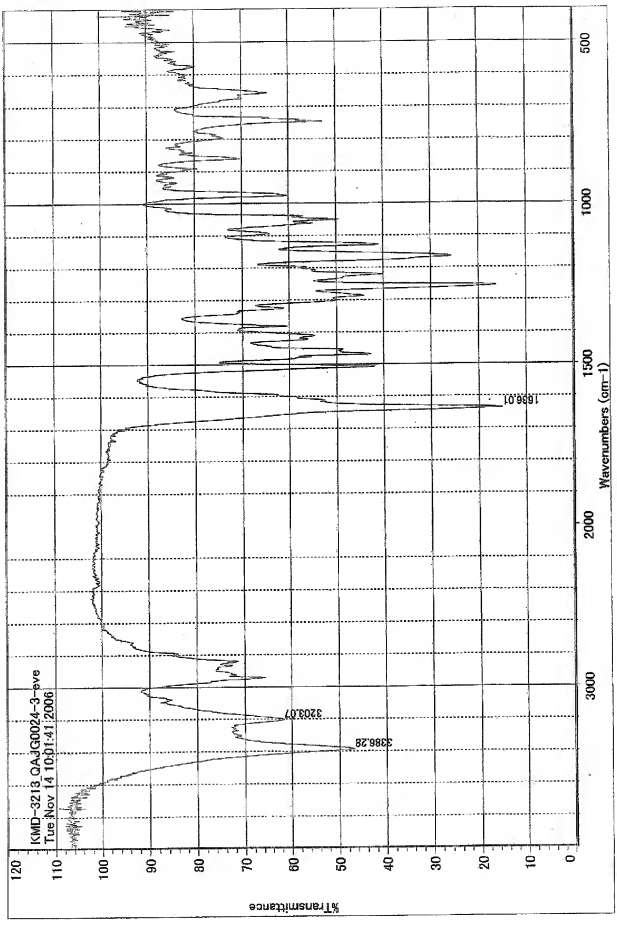
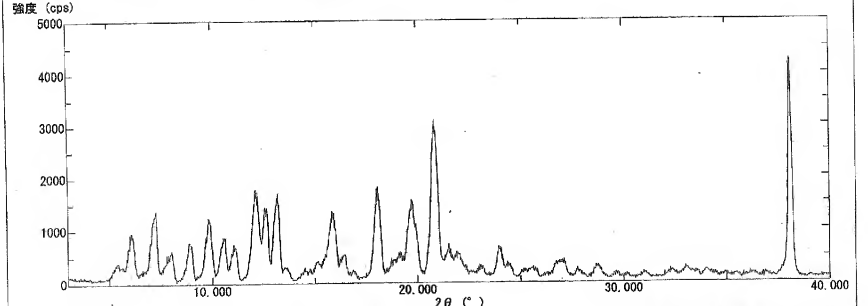
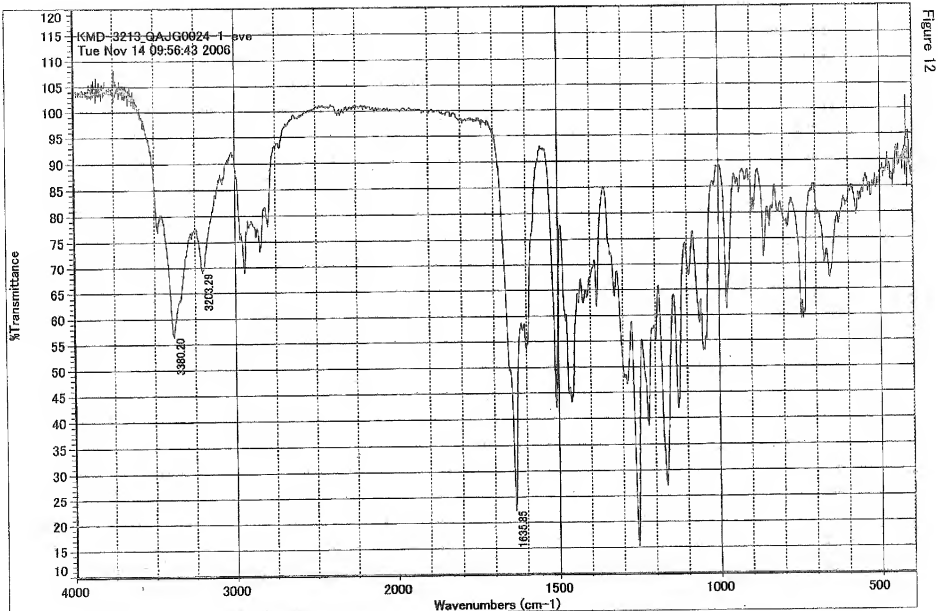

KMD-3213 QAJG0024-3-sve
Tue Nov 14 10:01:41 2006
%Transmittance
Wavenumbers (cm-1)
3386.28
3203.07
1636.01
120
110
100
90
80
70
60
50
40
30
20
10
0
3000
2000
1500
1000
500

Figure 11

生データ

ｻﾝﾌﾟﾙ名	: KWD-3213	ﾌｧｲﾙ	: 061109d03.raw	ｺﾒﾝﾄ	: QAJG0024-1-eve
測定者	: E.Tsuru	測定日	: 09-November-06 15:09:47		
ﾒﾓ	: 0.2mm				
X線	: Cu / 40 kV / 40 mA				
ｺﾞﾆｵﾒｰﾀ	: Ultima+ 水平ｺﾞﾆｵﾒｰﾀ				
ｱﾀｯﾁﾒﾝﾄ	: 6ｻﾝﾌﾟﾙﾁｪﾝｼﾞｬｰB型				
試料番号	: 3				
試料回転	: 回転	回転速度	: 120.000 rpm		
ﾌｨﾙﾀ	: 不使用	発散ｽﾘｯﾄ	: 1/2°	ﾓﾉｸﾛ受光ｽﾘｯﾄ	: 0.45mm
ｲﾝﾃﾞﾝﾄﾓﾉｸﾛ	: 不使用	発散縦制限ｽﾘｯﾄ	: 10mm		
ｶｳﾝﾀﾓﾉｸﾛﾒｰﾀ	: 固定ﾓﾉｸﾛﾒｰﾀ	散乱ｽﾘｯﾄ	: 1/2°		
ｶｳﾝﾀ	: ｼﾝﾁﾚｰｼｮﾝｶｳﾝﾀ	受光ｽﾘｯﾄ	: 0.15mm		
走査ﾓｰﾄﾞ	: 連続	ｽｷｬﾝ ｽﾋﾟｰﾄﾞ	: 4.000 °/min.	ｻﾝﾌﾟﾘﾝｸﾞ幅	: 0.020 °
走査軸	: 2θ/θ	走査範囲	: 3.000 ～ 40.000 °	θｵﾌｾｯﾄ	: 0.000 °
積算回数	: 1				




KMD-3213-GAJG0024-1-ave
Tue Nov 14 09:56:43 2006
%Transmittance
Wavenumbers (cm-1)
3380.20
3202.29
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120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
4000
3000
2000
1500
1000
500

Figure 12

ATTACHMENT 2

Journal of Chemical Engineering of Japan, Vol. 37, No. 10, pp. 1224–1231, 2004

Research Paper

The Effects of Impurities on Crystallization of Polymorphs of a Drug Substance AE1-923

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[2]*Fukui Research Institute, Ono Pharmaceutical Co., Ltd., 5-2, Yamagishi Technoport Mikuni-cho 1, Sakai-gun, Fukui 913-8538, Japan*

Keywords: Solvent-Mediated Transformation, Crystal Polymorphs, Drug Substance, Impurity, Inhibition of Transformation

The effects of seven kinds of impurities on the solvent-mediated transformation of crystal polymorphs of a drug substance AE1-923 were investigated. AE1-923 was developed for an indication of pollakiuria and pain. It has three polymorphs, namely the unstable A-form, the metastable B-form and the stable C-form. The impurities tested were the starting compounds, the reaction reagents and the intermediates in the synthesis of AE1-923. One of the impurities, an intermediate AE1-923ME (methylester of AE1-923), inhibited the solvent-mediated polymorph transformation of the B-form to the C-form, although the transformation of the A-form to the B-form was not inhibited. The other impurities had no effect on the two steps of polymorph transformation. The inhibitory effect of AE1-923ME that was evaluated by the overall transformation rate constant k_t exponentially increased with an increase in the concentration of AE1-923ME. AE1-923ME of 0.5 wt% completely suppressed the nucleation of the stable C-form crystals. However, crystal growth of the C-form was not inhibited.

Introduction

In the manufacturing of pharmaceuticals, polymorphism of crystals must be strictly controlled, because it significantly influences the bioavailability of a drug (Yokoyama *et al.*, 1981; Audran *et al.*, 1988). Impurity is one of the important factors in control of crystal polymorphism. Polymorphism is often affected by a trace amount of impurities (Davey, 1997; Domopoulou 1998). Many drug substances are synthesized through many reactions. It means that many kinds of chemicals, namely raw materials, intermediates, by-products, etc., may be remained in the solution of final product as a trace amount of impurities. Therefore, it is important to understand the effects of the impurities on appearance of crystal polymorphs. However, there have not been so many reports with respect to the effect of impurities on the crystallization of polymorphs of drug substances (Kuroda *et al.*, 1979; Kato *et al.*, 1981).

The aims of this report are to show and to discuss an example that an intermediate in the synthesis of a drug substance inhibited the solvent-mediated transformation of crystal polymorphs of a final product. In the present study, a drug substance AE1-923 (4-[[2-[*N*-(5-Methylfuran-2-sulfonyl)-*N*-isopropylamino]-5-(trifluoro-methyl) phenoxy] methyl] benzoic acid) was crystallized. AE1-923 was developed for an indication of pollakiuria and pain. It has three crystal polymorphs in a mixed solvent of ethanol and water, namely the unstable A-form, the metastable B-form and the stable C-form (Okamoto *et al.*, 2004). The A-form transforms to the C-form through the B-form and the transformation was promoted with an increase in the crystallization temperature and the ethanol ratio of an ethanol–water mixed solvent (Okamoto *et al.*, 2004).

1. Experimental

1.1 Materials and identification of polymorphs

AE1-923 and its synthetic intermediates shown in Figure 1 were provided by Ono Pharmaceutical Co., Ltd. The chemical purity of AE1-923 assayed by HPLC was higher than 99.7% and no impurity was contained at the level exceeding 0.1%. The chemical purities of other substances relating to the synthesis of AE1-923, which were used as impurities, were higher than 97%.

The other chemicals used were of the reagent grade and purchased from Wako Pure Chemical Industries, Ltd. Water used was of the manufacturing-grade guaranteed in the drug manufacturing specification. It

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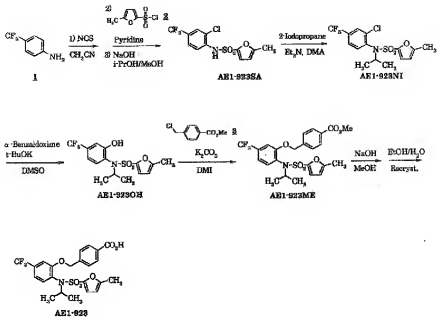

Fig. 1 Synthesis of AE1-923

was fully compliant with the drug manufacturing standard and its quality was guaranteed. An ethanol–water mixed solvent was prepared. In this paper, a mixed solvent containing *N* volume-% ethanol is expressed as *N*%-E solvent, for example 60%-E solvent.

Polymorphs were identified by IR spectroscopy (*in situ*-FTIR Model ReactIR 1000, ASI Applied systems Inc.). Figure 2 presents the characteristic IR absorption spectrum from 850 to 900 cm^{-1} of each crystal polymorph of AE1-923. The different polymorphs could be distinguished from their peak intensities, namely at 880, 870, and 860 cm^{-1} for the A-, the C-, and the B-forms, respectively. IR spectroscopy was also used to determine the composition of the polymorphs, where the limit of detection of polymorph was approximately 5%.

1.2 Solubility

Using the *in situ*-FTIR system (Okamoto *et al.*, 2004), solubility of three kinds of polymorphic crystals of AE1-923, namely the A-, B-, and C-form crystals were measured in a 60%-E solvent. The solubility of crystals of an intermediate compound AE1-923ME (methylester of AE1-923) was also measured in the same condition.

1.3 Crystallization and effects of impurities on the solvent-mediated transformation of the A-form to the C-form

The poor solvent crystallization of AE1-923 was carried out in the presence and absence of impurities. A jacketed cylindrical glass vessel of 500 mL with an agitator was used as a crystallizer (Okamoto *et al.*,

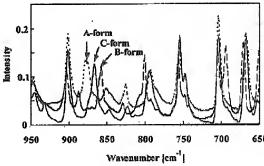

Fig. 2 IR spectra of three polymorphs of AE1-923 crystals

2004). The crystallization was separated into three steps, namely the first step was the exclusive formation of the A-form crystals not containing the B-form and the C-form crystals, the second step was the transformation of the A-form to the B-form, and the third step was the transformation of the B-form to the C-form.

First, the unstable A-from crystals were exclusively precipitated as follows (Okamoto *et al.*, 2004). 105 mL of 50%-E solvent was placed in the crystallizer at 0°C. 15 g of AE1-923 was separately dissolved in 75 mL of ethanol at 35°C. The whole solution was added to the solvent in the crystallizer under stirring at 300 rpm. The precipitation of the A-form crystals was continued for 30 min, where the temperature of

coolant circulating through the jacket was controlled at 0°C and the working concentration of ethanol was 70%. The A-form crystals did not transform to the B-form during the precipitation. We separately confirmed no transformation to the B-form within at least 24 h. No transformation is due to a low crystallization temperature (Okamoto *et al.*, 2004). Second, the solvent-mediated transformation of the A-form to the B-form was started as follows. The ethanol concentration of slurry of the A-form crystals prepared above was decreased to prevent the direct and uncontrolled rapid transformation to the stable C-form. Namely, 7.5 mL of ethanol and 37.5 mL of water were added to the slurry for adjusting the ethanol concentration to 60%. Then the temperature of the slurry was raised to 45°C. Part of the slurry was pipetted at regular intervals and filtered with a 0.45 μm membrane filter. The crystals recovered were dried at 40°C under reduced pressure and their crystal polymorphs were analyzed by FTIR. Third, the solvent-mediated transformation of the B-form to the C-form was started by raising the slurry temperature of the B-form crystals from 45 to 60°C.

The effects of impurities on the solvent-mediated transformation of AE1-923 crystals were examined as follows. Seven kinds of impurities shown in Figure 1 were used, namely the starting material (1), the reaction reagents (2, 3) and the reaction intermediates, AE1-923SA, AE1-923NI, AE1-923OH, AE1-923ME. An impurity was added to the AE1-923 solution before mixing with 105 mL of 50%-E solvent placed in the crystallizer. The impurity concentration was 0.5 wt% on a basis of the weight of AE1-923 used. Precipitation of the A-form crystals and the transformation of the A-form to the B-form and the transformation of the B-form to the C-form were evaluated.

1.4 The effect of AE1-923ME on the solvent-mediated transformation of the B-form to the C-form

Since AE1-923ME inhibited the solvent-mediated transformation of the B-form to the C-form as described later, several experiments were conducted to understand which processes were inhibited by AE1-923ME among three process of the transformation, namely dissolution of the B-form crystals, nucleation and crystal growth of the C-form crystals.

First, the dissolution rate of the B-form crystals was compared between in the absence and the presence of AE1-923ME. A 225 mL of 60%-E solvent was placed in a glass vessel of 500 mL and raised to 60°C. Then 15 g of the B-form crystals of AE1-923 was added under stirring at 300 rpm. The change in the concentration of AE1-923 was continuously measured with an *in situ*-FTIR system. When dissolution rate was measured in the presence of AE1-923ME, 75 mg (0.5 wt%) of AE1-923ME was dissolved in the solvent.

Second, in order to understand the effect of AE1-923ME on nucleation and crystal growth of the C-form crystals, the effect of the timing of impurity addition on the solvent-mediated transformation was investigated. 0.5 wt% of AE1-923ME was added to the slurry of the B-form crystals before or after starting the transformation of the B-form to the C-form.

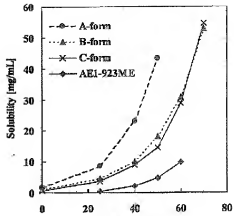

Fig. 3 Solubility curves of AE1-923 crystals and AE1-923ME in 60%-E solvent

Third, the effect of AE1-923ME on the growth of the C-form crystals was investigated by using seed crystals of the C-form in the presence of AE1-923ME. The C-form seed crystals of 24 wt% against the potential amount of precipitation of AE1-923 were added to the slurry of the B-form pre-incubated at 60°C for 2 h in the presence of 0.5% AE1-923ME. Two kinds of seed crystals differing in size were used, namely 16 and 1.4 μm in average particle diameter. The latter was prepared by grinding the former. The particle size was determined by the laser reflection method (FBRM, Lasentec Co. Ltd.). Other crystallization operation was the same as that mentioned above.

2. Results and Discussion

2.1 Identification of the impurity affecting the solvent-mediated transformation of the A-form of AE1-923 crystals to the C-form

AE1-923 consists of a benzoic derivative containing trifluoromethylphenyl group and a methylfuransulfonyl group, whose synthesis scheme is shown in Figure 1. Crude product was obtained by crystallization after hydrolysis of AE1-923ME. All intermediates of AE1-923 were crystalline solids.

As described above, AE1-923 has three crystal polymorphs in a ethanol–water mixed solvent, namely the unstable A-form, the metastable B-form and the stable C-form. The solubility of each crystal polymorph in 60%-E solvent is presented in Figure 3. Each value was an average of three experimental data that agreed

Table 1 The effects of impurities on the solvent-mediated transformation of AE1-923ME crystals

Impurities	Effect on the precipitation**	Effect on the transformation**	
(0.5 wt%)*	A-form	A-form → B-form	B-form → C-form
Compound 1	×	×	×
Compound 2	×	×	×
Compound 3	×	×	×
AE1-923SA	×	×	×
AE1-923NI	×	×	×
AE1-923OH	×	×	×
AE1-923ME	×	×	○

*the ratio of impurities to AE1-923
**×, not affected; ○, inhibited

within a 2% error. IR analysis confirmed that the transformation of crystal polymorphs did not proceed during the measurement of solubility. The solubility of the unstable A-form was two or three times larger than that of the other two polymorphs. The solubility of the B-form is close to that of the C-form. The solubility curves of the B- and C-forms showed an enantiotropic system. Namely the solubility curves of the two forms crossed at a certain temperature near 65°C.

When purified AE1-923 was used in the poor solvent crystallization, the reproducible experimental data were always obtained. For example, in the crystallization at 40°C in 60%-E solvent, the consecutive transformation of the A-form to the C-form via the B-form was always completed within 3 h (Okamoto *et al.*, 2004). However, when crude AE1-923 was used, the crystallization was not reproducible. Namely, the recovery of each polymorph was different case by case. For example, when the crystallization was carried out in 50%-E solvent at 40°C for 5 h, the B-form ratio of product crystals changed between 0.1 and 1.0 (data also not shown). The B-form ratio of 1.0 means that the solvent-mediated transformation of the B-form to the C-form did not progress. This result suggested that certain impurities inhibited the solvent-mediated transformation of the B-form to the C-form.

We attempted to identify the impurities affecting the solvent-mediated transformation of AE1-923 polymorphs. The impurities should be the chemicals included in the synthesis process shown in Figure 1. The substances examined as impurity were 7 kinds of chemicals listed in Table 1. Their effects on the consecutive solvent-mediated transformation of the A-form to the C-form via the B-form were examined. First, as mentioned in the experimental section, the A-form crystals were precipitated by poor solvent operation in the presence of impurities and then the consecutive transformation to the C-form crystals was started by adding ethanol to the slurry of the A-form crystals and raising temperature to 45°C. The experimental results obtained for 7 kinds of impurities were summarized in Table 1. All impurities showed no effect on the precipitation of the A-form crystals as long as it was evaluated from the amount of the A-form crystals recovered. Namely, in all cases, the whole amount of A-form crystals potentially expected from the solubility was recovered after 30 min-crystallization. The transformation of the A-form to the B-form also was not affected by any impurity examined. Namely, in all cases, the A-form crystals completely transformed to the B-form crystals through 30 min-incubation of the A-form crystals at 45°C in 60%-E solvent. The transformation of the B-form to the C-form was inhibited by an intermediate AE1-923ME as shown in Table 1 and Figure 4. AE1-923ME is a reaction precursor of AE1-923. 0.5 wt% of AE1-923ME completely disturbed the transformation of the B-form to the C-form. This result suggested that the fluctuation of the B/C polymorph ratio obtained in the crystallization of crude AE1-923 was caused by a trace of AE1-923ME remained in the crystallization solution as an impurity. Indeed, as Figure 2 also shows, the solubility of AE1-923ME is high enough to contaminate a crystallization solution.

2.2 The effect of the AE1-923ME concentration and temperature on the solvent-mediated transformation of the B-form to the C-form

The effect of the concentration of AE1-923ME on its inhibitory effect in the transformation of the B-form to the C-form was investigated at 60°C using 60%-E solvent. Since the transformation of the A-form to the B-form was not influenced by AE1-923ME at all as mentioned above, the transformation was started from the A-form crystals to fix the starting condition of the transformation of the B-form to the C-form as described in the experimental section. The concentration of AE1-923ME was changed from 0.05 to 0.5 wt% and the

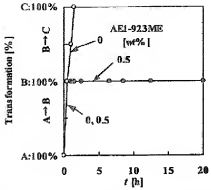

Fig. 4 The effects of AE1-923ME on the transformation of the A-form to the B-form and that of the B-form to the C-form in 60%-E solvent at 45°C

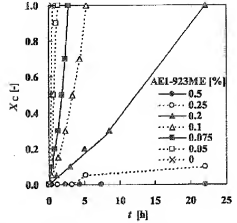

Fig. 5(a) The effects of the AE1-923ME concentration on the rate of transformation of the B-form to the C-form in 60%-E solvent at 60°C

inhibitory effect was evaluated by a time-dependency of the weight ratio of the C-form crystals, X_C, defined by Eq. (1),

$$X_C = W_C/(W_B + W_C) \tag{1}$$

where W_B and W_C are the weights of the B-from crystals and the C-form crystals contained in product crystals, respectively. In Eq. (1), the weight of the A-form crystals is not included, because all the A-form crystals transformed to the B-form crystals.

Figure 5(a) presents changes in X_C. Usually, the transformation time t is originated at the beginning of crystallization of the metastable polymorph, but in the present work the transformation time "zero" was set at the time when the temperature began to increase to 60°C to start the transformation as mentioned in experimental section. Figure 5(a) shows that the transformation started without any waiting time and that the larger the concentration of AE1-923ME, the more strongly the transformation was inhibited, while the transformation was completed within 30 min in the absence of AE1-923ME. AE1-923ME of 0.5 wt% completely inhibited the transformation.

Quantitative evaluation for the solvent mediated transformation of the B-form to the C-form was attempted. A solvent-mediated transformation consists of three processes: i) the nucleation of the stable form; ii) the crystal growth of the stable form; iii) the dissolution of the metastable form. When the crystal growth of the stable form is a rate-determining step and the growth rate is proportional to the crystal surface area, changes in the weight ratio of the C-form crystals, X_C, can be expressed by,

$$dX_C/dt = kX_C^{2/3}f(x) \tag{2}$$

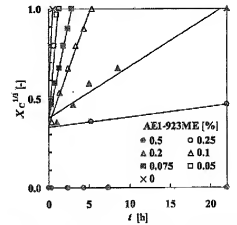

Fig. 5(b) Rearrangement of the data presented in Figure 5(a). Relationship between $X_C^{1/3}$ and transformation time t in the solvent-mediated transformation of the B-form to the C-form

where t is the transformation time (Sudo *et al.*, 1991). The k is the rate constant and $f(x)$ is the driving force for crystal growth of the C-form. For instance, $f(x)$ may be the n-th powered ΔC that is the difference in solubility between the B-form and the C-form. From the integration of Eq. (2), we obtain,

$$X_C^{1/3} = k_s(t - \theta) \qquad 0 < t < \theta, \quad X_C = 0 \tag{3}$$

where k_s is the overall transformation rate constant and equal to $kf(x)/3$. The θ is the waiting time for nucleation of the stable C-form. Since in the present work, no waiting time was observed, θ should be zero. The

Table 2 Dependency of the overall transformation rate constant k_e on the concentration of AE1-923ME at 50 and 60°C

Impurity [wt%]*	k_e [h^{-1}]	
	AE1-923ME	
	50°C	60°C
0	0.28	0.83
0.05	—	0.51
0.075	—	0.21
0.1	0.077	0.12
0.2	0	0.029
0.25	—	0.006
0.5	—	0

*based on the concentration of AE1-923

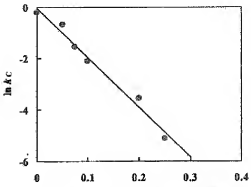

Fig. 6 Relationship between k_e at 60°C and the concentration of AE1-923ME. Straight line: $k_e = 0.86\exp(C_{imp})$, where C_{imp} is the concentration of AE1-923ME

rate of transformation varies depending on the agitation rate and temperature (Maruyama *et al.*, 1999), and contamination with impurities as described in the present work. Those influences are expressed in the overall transformation rate constant, k_e. This equation has been frequently used to express the transformation rate of polymorph (Maruyama and Ooshima, 2000). Figure 5(a) was rearranged by Eq. (3) and the results were presented in Figure 5(b). Linear relationships between $X_c^{1/3}$ and t were obtained. The linear relationship indicates that the rate-determining step of the transformation is crystal growth of the stable C-form crystals. However, those straight lines did not concentrate in the origin expected from no waiting time for nucleation of the C-form crystals (namely, θ = zero), but may concentrate in one point on the vertical axis ($X_c^{1/3} = 0.35$). The value of 0.35 in $X_c^{1/3}$ corresponds to 0.042 in X_c. Since the value is negligibly small, we estimate that this may be caused by experimental errors. The possible errors include an analytical error of polymorphs by IR spectroscopy and a possibility that the B-form crystals might have contained the C-form crystals less than 5 wt% before starting the transformation of the B-form. Unfortunately we cannot discuss the contamination of the C-form crystals less than 5 wt%, because the limit of detection of polymorph was approximately 5 wt% as described in the experimental section. The slope of the straight line corresponds to the overall transformation rate constant k_e. In Table 2, the values of k_e determined from each straight line were tabulated. The data were represented in Figure 6. The k_e values decreased with an increase in the concentration of AE1-923ME, but the decrease was not in direct proportion of the concentration of AE1-923ME, but in exponential.

The k_e values obtained at 50°C in 60%-E solvent were also tabulated in Table 2. Those data show that the transformation at 50°C is slower than that at 60°C and the slow transformation is more strongly inhibited by AE1-923ME. Namely the transformation at 50°C is completely inhibited by 0.2 wt% of AE1-923ME, while 0.5 wt% at 60°C.

2.3 Determination of the process inhibited by AE1-923ME in the solvent-mediated transformation of the B-form to the C-form

As mentioned above, the solvent-mediated transformation of the B-form to the C-form proceeds through three processes, the nucleation of the stable C-form, the crystal growth of the C-form and the dissolution of the metastable B-form. Whichever the process is inhibited by AE1-923ME, the solvent-mediated transformation will not proceed. We examined which process is inhibited by AE1-923ME.

2.3.1 Dissolution of the metastable B-form crystals

Figure 7 presents the dissolution curves of the B-form crystals in the presence and absence of 0.5 wt% of AE1-923ME. The dissolution rate was not affected by AE1-923ME. The solution concentration in the presence of 0.5 wt% of AE1-923ME reached the solubility of the metastable B-form crystals because of no transformation of the B-form to the C-form. On the other hand, the dissolution in the absence of AE1-923ME stopped at the solubility of the C-form crystals. It may be explained with the fact that a circumstance in the absence of AE1-923ME is favorable to the C-form. Indeed polymorph of the crystals recovered after 30 min was the C-form. At any rate, the dissolution test suggested that AE1-923ME inhibits the nucleation and/or crystal growth of the C-form.

2.3.2 The effect of AE1-923ME on the nucleation and growth of the C-form crystals

In order to clarify the effect of AE1-923ME on the nucleation and growth of the C-form crystals, an experiment was conducted, where the timing of AE1-923ME addition to the slurry

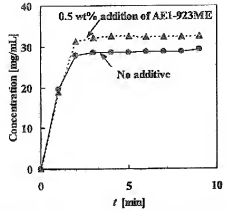

Fig. 7 Dissolution of the B-form crystals at 60°C in the presence and absence of 0.5 wt% AE1-923ME

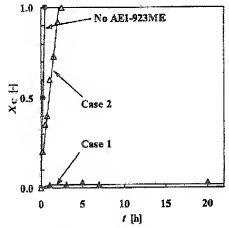

Fig. 8 The effect of the timing of the addition of AE1-923ME to the slurry of the B-form crystals in the transformation of the B-form to the C-form in 60%-E solvent at 60°C. Addition timing: Case 1, before the B-form crystals began to transform to the C-form crystals; Case 2, on the way of the transformation

of the B-form crystals was changed. First, AE1-923ME was added before the B-form crystals began to transform to the C-form crystals (Case 1). Another addition was carried out on the way of transformation (Case 2). The experimental results were presented in Figure 8. When AE1-923ME of 0.5% was added before the B-form crystals began to transform to the C-form crystals, the transformation of the B-form crystals never proceeded. On the other hand, when AE1-923ME was added after 20%-transformation, the transformation of the B-form crystals continued to proceed, although the

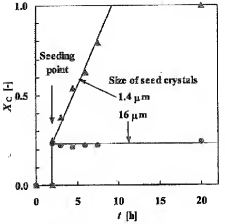

Fig. 9 The effect of the C-form seed crystals on the transformation of the B-form crystals in the presence of AE1-923ME in 60%-E solvent at 60°C

transformation rate was slightly decreased. These results mean that AE1-923ME does not inhibit the crystal growth of the C-form but preferentially inhibits the nucleation of the C-form crystals. In order to confirm this, another experiment was conducted as mentioned in the experimental section. Figure 9 shows the effect of the C-form seed crystals on the transformation of the B-form crystals in the presence of AE1-923ME. When the large seed crystals were used, the transformation of the B-form did not proceed. However, when the small seed crystals were used, the B-form crystals completely transformed to the C-form crystals, although the transformation rate was slow. This experiment confirmed the conclusion obtained in Figure 8 that even when the C-form crystals are present in solution, their growth proceeds, regardless of the presence of AE1-923ME. However, it is unknown why the large crystals were not active as seed crystals. Since as long as observed with an optical microscope, size of the C-form crystals obtained using small seed crystals was almost the same as that of the crystals obtained in the absence of AE1-923ME, namely about 16 μm, the limitation of growth may be present.

Figure 10 shows the Arrhenius plots of the overall transformation rate constants obtained in the absence and presence of 0.1 wt% AE1-923ME. The activation energy was determined to be 75.0 kJ/mol. Since the rate-determining step of the transformation is the growth of the C-form crystals, the activation energy determined should be that of the crystal growth of the C-form. The same value was obtained regardless of the absence and presence of AE1-923ME. This is another evidence that AE1-923ME does not inhibit the growth of the C-form crystals.

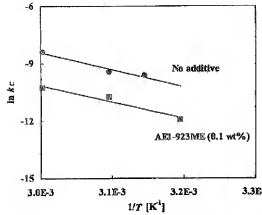

Fig. 10 Arrhenius plots of the overall transformation rate constants obtained in the absence and presence of 0.1 wt% AE1-923ME

Conclusions

The effect of impurities on the solvent-mediated transformation of crystal polymorphs of a drug substance AE1-923 was investigated. AE1-923 was developed for an indication of pollakiuria and pain. It has three polymorphs, namely the unstable A-form, the metastable B-form and the stable C-form.

An intermediate, AE1-923ME, in the synthesis of AE1-923 inhibited the transformation of the B-form crystals to the C-form crystals, but the transformation of the A-form to the B-from was not inhibited. As mentioned above, a solvent-mediated transformation proceeds through three processes, the nucleation of the stable form, the crystal growth of the stable form and the dissolution of the metastable form. AE1-923ME of 0.5 wt% completely inhibited the nucleation of the stable C-form crystals. The results shown in Figures 8, 9, and 10 suggested that AE1-923ME does not inhibit the crystal growth of the C-form.

The inhibitory effect of AE1-923ME was evaluated by the overall transformation rate constant k_v. As a result, it was found that the inhibitory effect of AE1-923ME exponentially increases with an increase in its concentration as shown in Figure 6. As long as the inhibitory effect was evaluated with a minimum concentration of AE1-923ME at which the transformation of the B-from to the C-form was completely inhibited, the inhibitory effect at 50°C was larger than that at 60°C.

Acknowledgment

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Nomenclature

$f(x)$	=	driving force for crystal growth of the C-form	[—]
k	=	transformation rate constant	[h^{-1}]
k_v	=	overall transformation rate constant	[h^{-1}]
t	=	transformation time	[h]
W_b	=	weight of the B-from crystals contained in product crystals	[kg]
W_c	=	weight of the C-from crystals contained in product crystals	[kg]
X_c	=	weight ratio of the C-form crystals defined by Eq. (1)	[—]
θ	=	waiting time	[h]

Literature Cited

Audran, M., Y. Lasserre, J. Rambaud, B. Pauvert, G. Berge and L. Maury; "Comparative-Study of *in vitro* Dissolution and Bone Absorption of Sulfamethadine and One of Its Solvates," *Pharm. Acta Helv.*, **63**, 343–346 (1988)

Davey, R. J., N. Blagden, G. D. Potts and R. Docherty; "Polymorphism in Molecular Crystals: Stabilization of a Metastable form by Conformational Mimicry," *J. Am. Chem. Soc.*, **119**, 1767–1772 (1997)

Domopoulou, A., A. Michaelides, S. Skoulika and D. Kovala-Demertzi; "Crystallization of an Unstable Molecular Compound Using a Tailor-Made Additive," *J. Crystal Growth*, **191**, 166–168 (1998)

Kato, Y., Y. Okamoto, S. Nagasawa and T. Ueki; "Solubility of a New Polymorph of Phenobarbital Obtained by Crystallization in the Presence of Phenytoin," *Chem. Pharm. Bull.*, **29**, 3410–3413 (1981)

Kuroda, K., T. Yokoyama, T. Umeda, Y. Kita, A. Konishi and T. Kuroda; "Studies on Drug Nonequivalence VIII. Solubilities of Polymorphs and Hydrate of Mercaptopurine," *Yakugaku Zasshi*, **99**, 745–751 (1979)

Maruyama, S. and H. Goshima; "Crystallization Behavior of Taltirelin Polymorphs in a Mixture of Water and Methanol," *J. Crystal Growth*, **213**, 239–245 (2000)

Maruyama, S., H. Goshima and J. Kato; "Crystal Structures and Solvent-Mediated Transformation of Taltirelin Polymorphs," *Chem. Eng. J.*, **75**, 193–200 (1999)

Okamoto, M., M. Hamano and H. Goshima; "Active Utilization of Solvent-Mediated Transformation for Exclusive Production of Metastable Polymorph Crystals of AE1-923," *J. Chem. Eng. Japan*, **37**, 95–101 (2004)

Sudo, S., K. Sato and Y. Harano; "Growth and Solvent-Mediated Phase Transition of Cimetidine Polymorphic Forms A and B," *J. Chem. Eng. Japan*, **24**, 628–632 (1991)

Yokoyama, T., T. Umeda, K. Kuroda, T. Kuroda and S. Asada; "Studies on Drug Nonequivalence X. Bioavailability of 6-Mercaptopurine Polymorphs," *Chem. Pharm. Bull.*, **29**, 194–199 (1981)

ATTACHMENT 3

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Polymorphism in Molecular Crystals: Stabilization of a Metastable Form by Conformational Mimicry

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Abstract: A combined modeling and experimental strategy has been applied to the problem of stabilisation of a metastable conformational polymorph. For the first time additives have been successfully selected which by virtue of their conformation are able to selectively inhibit the appearance of the stable β polymorph of L-glutamic acid and hence stabilize the metastable α structure.

Introduction

Polymorphism, the ability of a molecule to adopt more than one crystal structure, is fundamental to solid state chemistry[1] and is found in many classes of molecular materials, for example, triglycerides, saturated and unsaturated fatty acids, alkanes, aromatic π-bonded systems, amino acids, carboxylic acids, and amides. It is thus of importance across a wide range of industries including pharmaceuticals, healthcare, agrochemicals, pigments, dyestuffs, and foods. Recent developments in computational techniques coupled with increased appreciation and parameterization of intermolecular interactions have led to the availability of commercial software for the prediction of polymorphic crystal structures from molecular structures.[2−4] In terms of the development of robust processes for isolating polymorphic materials a structural approach, however, is limited since it neglects the vital role of kinetics in determining the appearance of polymorphic structures, a factor which Ostwald recognized almost a century ago in his famous Law of Stages.[5,6] Technologically this is crucial since these structural variants exhibit different physical properties which are reflected in crystal morphology, optical characteristics, mechanical properties, and chemical reactivity. Thus solid−liquid separation, comminution, solubility, particle flow, and formulation characteristics will all be polymorph dependent.[7,8] In some instances this allows polymorphism to be exploited such that the structure with properties appropriate for a particular formulation is isolated. In other cases the isolation of a new polymorph can threaten product specifications and radically change the status quo in the patent arena as in the recent case of Zantac.[9] Despite this our ability to manipulate the kinetic processes occurring during crystallization of polymorphic systems is extremely poor and limits the level of process control available in high performance specialty chemical production. A recent analysis by Bernstein and Dunitz[10] has highlighted this issue by documenting a number of so-called "disappearing polymorphs", i.e., sudden appearances of new structures or the unexplained disappearances of existing ones. Such examples are almost certainly mirrored (although not documented) by industrial practice and one can only speculate at the disastrous consequences of a sudden unexplained switch of polymorph during the isolation of a high value specialty product.

Previous work aimed at controlling the polymorphic outcome from crystallization processes has shown, on the one hand, how impurity induced twinning can inhibit a solid state transformation and hence lead to the kinetic stabilization of a metastable polymorph[11] and, on the other hand, how additives may be used to inhibit the nucleation of unwanted polymorphic structures in crystallization from solution.[12,13] The design of additives for the latter application was facilitated by gross differences in symmetry, one of the polymorphs belonging to a centric and the other to a noncentric space group. The current work explores further the use of additives in polymorph control by addressing the issue of conformational polymorphism.[14] The results presented here, on L-glutamic acid, demonstrate, for the first time, the possibility of designing additive molecules to selectively inhibit the crystallization of the more stable polymorph on the basis of conformational recognition, allowing kinetics to dominate the crystallization process and leading to the stabilization of a metastable phase.

Ostwald's Law of Stages, L-Glutamic Acid, and Design Strategy

Ostwald's Law of Stages[5] states simply that "when leaving an unstable state, a system does not seek out the most stable

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⊗ Abstract published in *Advance ACS Abstracts*, January 15, 1997.

(1) Desiraju, G. R. *Crystal Engineering*; Materials Science Monographs, 54; Elsevier: Amsterdam, 1989.

(2) Gavezzotti, A. *Acc. Chem. Res.* **1994**, *27*, 309−314.

(3) Gavezzotti, A.; Filippini, G. *J. Am. Chem. Soc.* **1996**, *118*, 7153−7157.

(4) Karfunkel, H.; Gdanitz, R. J. *J. Comput. Chem.* **1992**, *13*, 1171−1183.

(5) Ostwald, W. *Grundriss der Allgemeinen Chemie*, Leipzig, 1899.

(6) Cardew, P. T.; Davey, R. J. *Tailoring of Crystal Growth*; Institution of Chemical Engineers, UK (North West Branch), 1982; pp 1−7. Cardew, P. T.; Davey, R. J. *Faraday Discussions* **1993**, *No. 95*, 160−162.

(7) Davey, R. J. *Separation Technology The Next Ten Years*; Garside, J., Ed.; Institution of Chemical Engineers: UK, 1994; pp 73−95.

(8) Davey, R. J. *Manufacturing Chemist* **1990**, Nov, 25−29.

(9) Ranitidine hydrochloride, marketed by Glaxo plc under the tradename "Zantac" is known to be polymorphic. In a recent patent litigation (Glaxo Inc. (1993), Plaintiff V. Novopharm Ltd., Defendant, No. 91−759-CTV-% BO United States District Court for the Eastern District of California, Raleigh Division 1993 U. S. Dist. LEW/S 13928) Glaxo's patent protection based on this polymorphism has been challenged.

(10) Dunitz, J. D.; Bernstein, J. *Acc Chem Res.* **1995**, *28*, 193−200.

(11) Davey, R. J.; Maginn, S. J.; Andrews, S. J.; Buckley, A. M., Cottier, D.; Dempsey, P.; Rout, J. E., Stanley, D. R.; Taylor, A. *Nature* **1993**, *366*. 248−250.

(12) Staab, E.; Addadi, L., Leiserowitz, L.; Lahav, M. *Adv. Mater.* **1990**, *2*, 40−43.

(13) Weissbuch, I.; Leiserowitz, L.; Lahav, M. *Adv. Mater.* **1994**, *6*, 952 −956.

(14) Bernstein, J.; Hagler, A. T. *J. Am. Chem. Soc.* **1978**, *100*, 673−681.

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Figure 1. The conformation of L-glutamic acid in (a) the α and (b) the β crystal structure. The torsions τ_1 and τ_2 are defined by carbons 2,3,4,5 and 3,4,5,6, respectively and have values of 59.3 and 68.3° in the α structure and −171.5 and −73.1° in the β structure.

state, rather the nearest metastable state which can be reached with loss of free energy". It has been shown previously that this law has no general proof, rather it is a special case of nucleation and growth in a polymorphic system.[6] For a system in which two polymorphs, I and II, exist, adherence to this law requires that the initial mass fraction of crystals of form I in the product is close to unity. This is only true when the product of the crystal nucleation rate, J, and the kinetic coefficient for crystal growth, k, is lower for II, the more stable phase,[6] viz.

$$J_{\mathrm{II}}k_{\mathrm{II}}{}^3 \ll J_{\mathrm{I}}k_{\mathrm{I}}{}^3$$

This suggests that the appearance of different structures may be influenced by additives designed to interfere selectively with either the nucleation or growth rates of a particular phase.

In the case of L-glutamic acid two polymorphs are known, designated α and β. The crystal structures are both orthorhombic, $P2_12_12_1$, with $a = 1.0282$, $b = 0.8779$, $c = 0.7068$ nm and $a = 0.5159$, $b = 1.730$, $c = 0.6948$ nm, respectively,[15,16] and crystals form with distinct rhombic and needle-like morphologies. Molecules crystallize in their zwitterionic state with molecular packing of both forms dominated by intermolecular hydrogen bonding[17] and electrostatic interactions, the most significant difference residing in the molecular conformations adopted in the two structures. These are shown in Figure 1 which defines the two torsions, τ_1 and τ_2, and shows the relatively extended conformation from the β structure compared to the more twisted α conformer.

The phase diagram for the L-glutamic acid−water system has been measured previously[18] and is known to be monotropic with the β form the more stable at all measured temperatures. In agreement with Ostwald's Law it is known that crystallization results in the initial nucleation and growth of the metastable α phase at all temperatures. If these α crystals are separated from their mother liquor immediately after crystallization, the dry crystals are indefinitely stable: there is evidently no solid state route to the β structure. If no separation is performed, however, a solvent-mediated transformation takes place[18−20] in which α crystals dissolve and β crystals grow from the mother liquor in a process whose rate increases with temperature.[18]

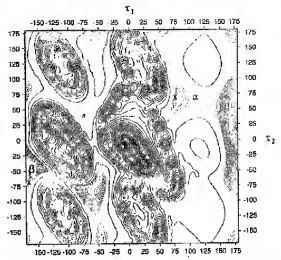

Figure 2. The calculated conformational map for L-glutamic acid with torsions τ_1 and τ_2 as defined in Figure 1. The location of α and β conformers are indicated. The TRIPOS force field was used, and the contour details are as follows: dashed lines −4.0 to +5.0 kcal/mol with contour spacing of 0.5 kcal/mol; solid lines +5.0 to +200 kcals/mol with contour spacing of 10 kcal/mol.

The overall objective of this work has been to select additives which by virtue of their *conformation* are able to selectively inhibit the crystallization of β and hence control the polymorphic outcome of L-glutamic acid crystallization yielding only α crystals. The strategy for achieving this objective has involved three separate tasks: firstly, simple conformational analysis aimed at establishing both the extent to which selected additives are likely to mimic α and β conformations and the degree to which the growth of L-glutamic acid itself is limited by the populations of appropriate conformers; secondly, the identification of the fastest growing faces of α and β crystals since to stabilize the α phase an additive must selectively bind to and inhibit at least the fastest growing faces of β crystals without affecting the fast growing faces of α crystals; and thirdly, an experimental protocol for testing the potency of additives in stabilizing the α polymorph relative to the more stable β structure.

Conformational Analysis

As a guide to the likely populations of molecules in α and β conformations, conformational analyses of L-glutamic acid and additives employed were carried out at the molecular mechanics level using both DREIDING[21] and TRIPOS[22] force fields. Published default parameters were used[21,22] together with AM1[23] charges. The resultant conformational minima were validated using semi empirical molecular orbital calculations within MOPAC.[23] In the case of L-glutamic acid conformational space, as defined by the torsions τ_1 and τ_2 (defined in Figure 1), was searched in 10° steps from 0 to 350°. The resulting map, Figure 2, demonstrates that molecular conformations corresponding to

(15) Lehmann, M. S.; Nunes, A. C. *Acta Crystallogr.* **1980**, *B36*, 1621−1628.
(16) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. *J. Cryst. Mol. Struct.* **1972**, *2*, 225−233.
(17) Bernstein, J. *Acta Crystallogr.* **1991**, *B47*, 1004−1010.
(18) Kitamura, M. *J Cryst. Growth* **1989**, *96*, 541−546.
(19) Kitamura, M.; Funahara, H. *J. Chem. Eng. Jpn.* **1994**, *27*, 124−126.
(20) Cardew, P. T.; Davey, R. J. *Proc. Roy. Soc. (London)* **1985**, *A393*, 415−428.
(21) Mayo, S. L.; Olafson, B.; Goddard, W. A. *J. Phys. Chem.* **1990**, *94*, 8897−8909.
(22) Clark, M.; Cramner, R. D.; Van Opdenbosch, N. *J. Comput. Chem.* **1989**, *10*, 982−1012.
(23) Stewart, J. J. P. MOPAC 6.0, QCPE No. 445, 1990.

Table 1. Conformational Data[a] for L-Glutamic Acid and Additive Molecules

molecule	$\Delta E_{torsion}$ between α and β conformers (kcal mol^{-1})	% in α conformation
L-glutamic acid	−0.2	45
glutaric acid	1	80
2-methylglutaric acid	1.5	90
transglutaconic acid	−7.5	0

[a] Calculated using DREIDING force field.

the α and β structures represent energy minima[24] connected by a low energy pathway involving rotation mostly around τ_1. Large areas of conformational space are unavailable, and it is clear that while the α conformation lies in a localized minimum the β conformation lies in valley running parallel to the τ_2 axis allowing it torsional freedom in this region at little energetic expense. The carboxylate groups of the additive molecules were fixed according to the α and β conformations and rotation around τ_1 and τ_2 employed to transform molecules between conformations. The torsional energy was monitored, and the barrier heights in each direction were estimated in order to evaluate the difference in torsional energy between forms. The results of this analysis are shown in Table 1 where the relative Boltzmann populations of the two conformational states have also been estimated. In the case of the L-glutamic acid zwitterion the β conformation is the more stable with an activation barrier of 2.6 kcal mol^{-1} for transforming α to β. This is consistent with the known solution chemistry of the system[22] and the crystallization characteristics discussed above in which it thus seems that conformational barriers are unlikely to be rate limiting and hence that molecules joining a growing surface of either phase are those already in the appropriate conformation. This is an important conclusion since it gives credence to the notion that additive selectivity may be based on conformational mimics of the two forms.

Additive Selection

The additives were selected on the basis of the morphological data which defined the fastest growing faces of each form (see below) and comprised four 1,5-dicarboxylic acids: glutaric ($HOOC(CH_2)_3COOH$), 2-methylglutaric ($HOOCCH(CH_3)(CH_2)_2COOH$), transglutaconic ($HOOCCH_2(HC{=}CH)COOH$), and trimesic (1,3,5-benzenetricarboxylic acid) acids, judged to have increasingly rigid conformations. Table 1 summarizes the results of the simple conformational analyses described above. These are consistent with this judgment, indicating the preference of glutaric and 2-methylglutaric acids for the α conformation, while decreased torsional flexibility imposed by the double bond predisposes transglutaconic acid to the β conformation. In the case of trimesic acid the rigid nature of the aromatic link precludes rotation along τ_1 so that the α conformation is inaccessible, and, as shown in Figure 3, trimesic acid mimics closely the β conformation. This is supported by Table 2 which compares the distances between carbon and oxygen atoms in the carboxylate groups of trimesic acid, taken from the crystal structure[16] with the equivalent distances in the two glutamic acid conformers. Thus, the overall predictions of the effects of these additives, based on the extent to which they are able to mimic the α and β conformations, are that glutaric and 2-methylglutaric acids should show minimal selectivity between the polymorphs with a possible preference for the α form, while transglutaconic and trimesic acids which are present exclusively in the β conformation should selectively inhibit the crystallization of the β phase, thus stabilizing the metastable α structure. Trimesic acid is expected to show an enhanced effect compared to transglutaconic acid both due to its increased torsional rigidity and its aromatic ring which will offer a greater steric barrier to crystal growth.

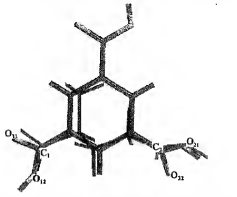

Figure 3. Comparison of trimesic acid and β glutamic acid conformations. Distances between labeled atoms are given in Table 2.

Table 2. Geometric Comparison of Carboxylate Separations in Trimesic and L-Glutamic Acids[a]

molecule	$-C_1\cdots C_5-$ distance (nm)	$-O_{11}\cdots O_{21}-$ distance (nm)	$-O_{12}\cdots O_{22}-$ distance (nm)
Trimesic acid	0.5	0.71	0.488
α-L-glutamic acid	0.382	0.575	0.335
β-L-glutamic acid	0.469	0.664	0.479

[a] See Figure 3 for atom numbering.

Experimental Section

Using previous work as a guide[18,19] experimental protocols were developed in which crystallization from aqueous solutions yielded crystals of the two polymorphic forms either as pure phases or mixtures. Thus pure α was prepared by seeding a 20 g/L aqueous solution at 18 °C, pure β was obtained by unseeded crystallization of a 35 g/L aqueous solution at 38 °C, and a mixture of forms was obtained from a 45 g/L solution crystallized at 48 °C. Crystallizations were carried out in thermostated glass vessels at the isoelectric pH (3.2) both unstirred and with gentle (magnetic) stirring. Additives (ex Sigma and Aldrich) were added prior to crystallization. For all the compositions used, the starting solutions were supersaturated with respect to both phases, and, as expected, in all experiments α was the first phase to appear. In order to assess the ability of additives to stabilize the α structure the overall $\alpha \rightarrow \beta$ transformation times were estimated at both 38 and 45 °C by sampling and using combined X-ray diffraction and optical microscopy.[18] A few transformation experiments were performed in an unstirred temperature controlled microscope cell in order to observe the process *in situ*.

Large (1 mm) crystals of each form were grown from unstirred solutions, and their morphologies were determined by a combination of X-ray oscillation photography and optical goniometry in order to define the crystal surfaces of interest and determine the morphological effects of additives.

Results

Pure Morphologies: Identification of Fastest Growth Directions. Figure 4a,b shows examples of α crystals grown at 18 °C and β crystals grown at 38 °C. α Crystals always grew as single well-formed rhombs, whilst β crystals tended to nucleate and grow as clusters of fragile needles. The indexed morphologies are shown diagrammatically and allow the

(24) Allinger, N. L.; Burkit, U. ACS Monograph No. 177; American Chemical Society: Washington DC, 1982.
(25) Hirayama, N.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 30−35.
(26) Ducharnp, D. J.; Marsh, R. E. *Acta Crystallogr.* **1969**, *B25*, 5−19.

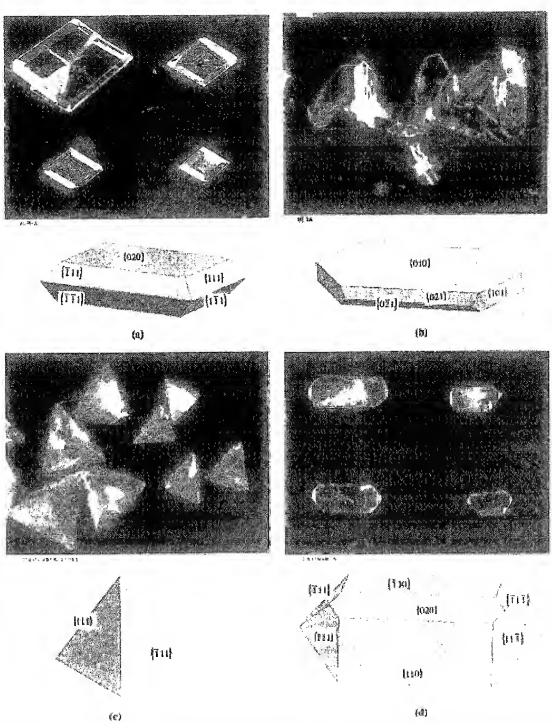

Figure 4. The morphologies (experimental and diagrammatic) of α and β crystals of L-glutamic acid: (a) α crystals grown from pure aqueous solution, (b) β crystals grown from pure aqueous solution, (c) α crystals grown in the presence of glutaric or methyl glutaric acids, (d) α crystals grown in the presence of transglutaconic or trimesic acids.

identification of the fastest growing crystallographic directions as {111} in α and {101} in β.

Conformational Discrimination and Selection of Additives. The molecular packings in these fast growing surfaces are shown in Figure 5a,b[27] and it is based on the information held within these surfaces that discriminatory additives were selected for the stabilization of the α form. This was based on the reasoning described above that to prevent the appearance of a particular polymorph it is essential to inhibit at least its fastest growth directions. In both sets of faces there are four molecular orientations with intermolecular hydrogen bonds between $-NH_3^+$, $-COO^-$, and COOH functionalities creating a two

(27) All packing diagrams were visualized using CERIUS, molecular modeling software, MSI/ Biosym Inc., Cambridge, UK.

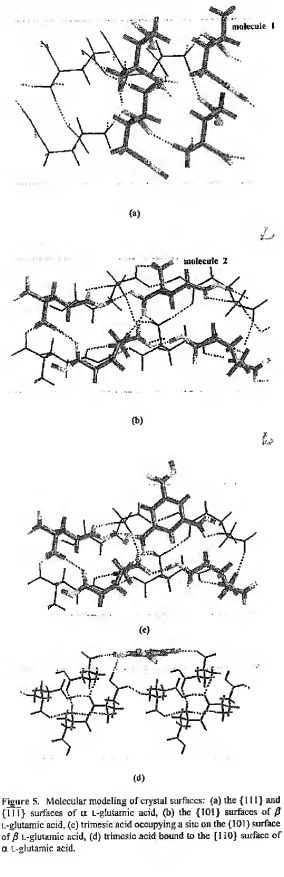

Figure 5. Molecular modeling of crystal surfaces: (a) the {111} and {$\bar{1}\bar{1}\bar{1}$} surfaces of α L-glutamic acid, (b) the {101} surfaces of β L-glutamic acid, (c) trimesic acid occupying a site on the (101) surface of β L-glutamic acid, (d) trimesic acid bound to the {110} surface of α L-glutamic acid.

Table 3. The Influence of Additives on the Appearance and Stability of the β Form of L-Glutamic Acid

additive	time for α to appear (min)	relative stability of α (−)[a]	aspect ratio of β crystals a:c (−)	length of β crystals along [001] (μm)
pure	5	1	1:4	40−65
glutaric acid	20	2	1:3	10−15
2-methylglutaric acid	30	3	1:5	10−15
transglutaconic acid	50	10	1:2	13−18
trimesic acid	35	1000	1:1	6−9

[a] The relative stability of α has been defined as time taken for 75% conversion to β in presence of additive/time taken for 75% conversion to β in pure solution.

dimensional network parallel to the surface. It is this feature which makes conformational discrimination possible since it precludes surfaces of one conformation from accepting molecules of the other and means that the requirement for any successful additive molecule is that it should have the appropriate conformation and on entering the surface should be capable of taking part in the hydrogen bonding network with minimum disruption. To achieve this the 1,5-dicarboxylic acids described above were selected as additives. Such molecules, lacking the protonated amino group, would clearly be capable of entering the growing surfaces only in sites at which the amino group plays little or no role in the in-plane hydrogen bonding networks. For the {111} and {$\bar{1}\bar{1}\bar{1}$} surfaces of α (which comprise two sets of nonequivalent Friedel pairs) only the molecule in position 1 (Figure 5a) on the {111} faces represents such a site, while for the {101} faces of β the equivalent site is defined by molecule 2 (Figure 5b). In both sites a 1,5-dicarboxylic acid of appropriate conformation could enter the surface with its carboxyl functionalities substituting for those of glutamic acid. The missing amino group would not be noticed until the next growth layer were laid down when its absence, or substitution for a bulkier moiety, would disrupt and inhibit growth. In this way it becomes clear that the 1,5-dicarboxylic acids selected, being of increasingly rigid and β-like conformation should be able to selectively prevent the growth of β crystals and hence stabilize the α phase. Figure 5c, constructed simply on the basis of a geometric fit, illustrates the expected incorporation of trimesic acid into the (101) face of the β structure, showing how the carboxylated phenyl ring points out into the solution to disrupt the addition of further L-glutamic acid molecules and inhibit growth. Further, those additives capable of accessing α conformations should influence only {111} and not {$\bar{1}\bar{1}\bar{1}$} faces of α crystals giving rise to polar morphologies.

Experimental Verification. To test these predictions experiments were performed using the protocols described above to determine the effects of these additives on the appearance, stability, and morphologies of the individual polymorphic forms. The results of experiments performed in stirred vessels at 38 °C with 10 mol% of additive are summarized in Table 3 which records four aspects of the crystallization process: the time taken for the initial appearance of α crystals, the stability of these α crystals in terms of their rate of transformation to the β structure, the a:c aspect ratio, and size of the final β crystals. A number of factors are clear from these data.

Firstly, all these additives have some influence on the crystallization of α with nucleation delayed significantly compared to pure solution. This influence was confirmed by examining the morphologies of α crystals grown at 18 and 38 °C in the presence of the additives. As expected, with glutaric and 2-methylglutaric acids crystals developed an increasingly polar morphology. At 10 mol% they appeared as pyramids (Figure 4c), in which only one set of the Friedel opposites {111} were present. This confirms that these molecules do indeed only access the surface site indicated in Figure 5a, replacing

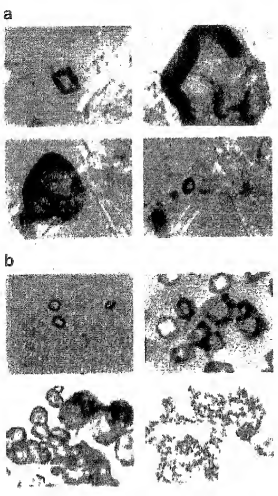

Figure 6. Time lapse micrographs of the $\alpha \rightarrow \beta$ solution mediated phase transition (a) (upper left, 20 min; upper right, 90 min; lower left, 150 min; lower right, 220 min) in pure solution and (b) (upper left, 35 min; upper right, 60 min; lower left; 1 week; lower right, 3 weeks) in the presence of 10% trimesic acid.

glutamic acid molecules by virtue of their ability to adopt α-like conformations. In the presence of transglutaconic and trimesic acids, however, α crystals displayed a morphology (Figure 4d) in which the previously unobserved {110} faces appeared. This unexpected result is consistent with the binding of these additive molecules to carboxylic acid groups of adjacent surface glutamic acid molecules whose separation (0.71 nm) can only be matched when the additive molecules adopt the β conformation. This is shown in Figure 5d for trimesic acid. Thus, on the basis of the morphological modification of α alone the expected conformational states of the additives are confirmed although their interactions with the α structure are more complex than predicted, being modified by binding to {110} surfaces.

Secondly it is clear from Table 3 that the major thesis of this study is proven, namely that additives which mimic the conformation of molecules in the thermodynamically stable structure are able to kinetically stabilize the metastable polymorph. Thus, in a pure, stirred, L-glutamic acid solution at 38 °C most of the α crystals have disappeared after 35 min to be replaced by β. This transformation time is equivalent to a relative stability of unity as defined by Table 3. With glutaric and 2-methylglutaric acids which can access both α and β conformations the selectivity between the polymorphs is limited, and this time is merely doubled. For transglutaconic and trimesic acids however, it is extended by an order of magnitude and for up to 3 weeks, respectively, as expected in view of their strong preference for the β conformation and the relatively larger steric barrier to crystal growth offered by trimesic acid. Figure 6 shows two sequences of time-lapse photomicrographs taken during the solution mediated $\alpha \rightarrow \beta$ transformation in pure unstirred solution and in a stirred solution containing 10% trimesic acid. For the pure system (Figure 6a) both rhombic, α, and needle, β, crystals are present after 20 min, and the sequential photographs focus on a central α rhomb which has grown to a size of about 100 μm after 90 min. Subsequent dissolution yields the situation after 220 min in which this α crystal has reduced in size and adopted a rounded morphology typical of a dissolving crystal, while the β crystals continue to grow. When trimesic acid is present (Figure 6b), no β needles are evident even after a week. The α crystals exhibit a modified morphology with the surface rounding due to dissolution only being evidence after 1 week. The final β product crystals are shown after 3 weeks to be small isometric crystals.

In the absence of stirring the effect of trimesic acid is dramatically enhanced with no evidence of β crystals after one month. The resulting β crystals show some decrease in size but no change in morphology in the presence of glutaric and 2-methylglutaric acids, while for transglutaconic and trimesic acids the a:c aspect ratio is decreased, and the crystal sizes are significantly smaller. This confirms that, as expected (Figure 5c) these additives attack the {101} surfaces, becoming more effective with increasing conformational rigidity. The decrease in size suggests that some changes in nucleation rate have taken place. Further studies on trimesic acid showed it to be active in preventing the appearance of β crystals for 1 week at levels as low as 0.1 mol% at 38 °C, while at 48 °C a 10% loading stabilizes α for a period in excess of 3 days.

Conclusions

Overall the results mirror well the predictions and serve as a powerful demonstration of the importance of molecular recognition at crystal surfaces in determining the outcome of the supramolecular assembly process operating in polymorphic systems. The possibility of using conformational mimicry to stabilize metastable structures of conformational polymorphs has been demonstrated for the first time and offers now a powerful tool in the development of robust processes in polymorphic systems. It is particularly gratifying and an indication of the predictive power of this strategy that the additives reported here were not found by a trial and error approach: they were the only ones selected for this task. Finally, it is noted that attempts to select additives which mimic the α conformation and hence lead to direct crystallization of β and nonadherence to Ostwald's Law were unsuccessful. Despite searching the Cambridge Crystallographic Database it did not prove possible to find a molecule with sufficient rigidity to mimic only the α conformation.

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